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Ultrastructural Study of X-ray Irradiated Spores of *Equisetum arvense* L.[1]

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ABSTRACT: Fresh spores of *Equisetum arvense* L. were irradiated for 5s, 15s, 30s or 60s, and examined with a transmission electron microscope. Alterations to the fine structure of the spore wall and the protoplasm are described. The exospore was more resistant to X-ray irradiation than to organic solvents, in particular diethylamine. The molecular structure of the spore wall was revealed by the X-ray effect. Resistance of the different layers of the sporomorphs to irradiation and organic solvents is important for understanding the complicated biopolymer system of the sporopollenin.

KEY WORDS: ***Equisetum*** **spore, Ultrastructure, X-ray effect.**

INTRODUCTION

The interesting morphological characteristics of the spores of *Equisetum* are known from the monographical elaboration of Milde (1865). Several light, transmission and scanning electron microscopical investigations have been carried out on these spores. Erdtman (1954) described the spore as a transitional type between alete and monolete spores. Welman (1970), p. 20 "Spores: cryptopolar, actinomorphic, spherical, alete." Erdtman and Sorsa (1971) described these spores as spheroidal, alete. Huang (1981) pointed out, that the spores of Equisetaceae are variable from alete, monolete to trilete in the same individual specimen. Ryabkova (1982) observed 2 - 3 apertural forms of these spores.

Fossil, elater-bearing spores were described and illustrated by Daugherty (1941), Wilson (1943), Good (1975, 1977), Good and Taylor (1974, 1975).

The transmission electron microscope has been used for different kinds of investigations. Gullvåg (1966) reviewed studies of the fine structure and of the spores and pollen grains. The protoplasm, primary storage products and the methodological investigation of the microbodies were discussed by Gullvåg (1968a,b, 1969, 1971). Positive catalase reaction was observed from the microbodies of spore cells of *E. arvense* by Olsen and Gullvåg (1973). Sporoderm development (sporogenesis) was described from *E. fluviatile* by Lehman, *et al.* (1984). Uehara and Kurita (1989) investigated spore wall morphogenesis of *E. arvense* using a TEM.

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Sitte (1963) established that the inner layer of the elaters is comprised of longitudinal cellulose microfibrils. The outer layer is composed of noncellulose polysaccharides. These two layers were also observed by Uehara and Kurita (1989). Uehara and Murakami (1995) investigated the arrangement of cortical microtubules during spore formation of *E. arvense* by immunofluorescence microscopy.

There have been a number of exospore studies using the TEM. Lugardon (1969, 1975) pointed out that two layers may be distinguished in the wall. Moreover the aperture was described based on the TEM data (Lugardon, 1976). Nilsson *et al.* (1977) wrote that the wall consists of thin, undulate perispore, a two layered partly lamellar exospore, endospore and cytoplasm. Later, Lugardon (1986) described the ultrastructure of the aperture in detail. Kedves (1979) used the light microscope and SEM to study several species (*E. arvense* L., *E. bogotense* H.B.K., *E. fluviatile* L., *E. palustre* L., *E. pratense* Ehrh., *E. silvaticum* L., *E. telmateia* Ehrh., *E. giganteum* L., *E. ramosissimum* Desf. subsp. *ramosissimum*, *E. ramosissimum* Desf. subsp. *debile* (Roxb.) Hauke, *E. myriochaetum* Schlecht *et* Cham., *E. laevigatum* A., Br., *E. hiemale* L., *E. variegatum* Schleich., *E. scirpoides* Michx.) The apertural area was observed with both the LM and the SEM.

The results of the high temperature effect were published by Kedves *et al.* (1991), and Kedves (1994). During the partial dissolution of the sporomorphs by organic solvents we obtained surprising results (Kedves and Gáspár, 1994a, b). For example the diethylamine dissolved the exospore of *Equisetum arvense*, but the elaters were extremely resistant. The perispore dissolved later than the exospore. Regarding the LM results of the X-ray irradiated spores (Kedves and Gáspár, 1994c, 1995) minor alterations were observed only. TEM of partially degraded spores of *Equisetum arvense* by 2-aminoethanol and $KMnO_4$ solution discovered highly organized biopolymer structures. The arrangement of globular units in pentagons was established (Kedves and Winter, 1988).

The aim of this paper is to present the results of *Equisetum arvense* spores after four different doses of irradiation in comparison to the previous results in this subject, particularly the solubility of the thick exospore in diethylamine. Alterations in the ultrastructure of the different layers of the spore wall are important for determining the relative resistance of the sporoderm layers of the molecular structure of the sporopollenin. These data may be useful in the investigation of fossil sporomorphs and taphonomical processes during fossilization.

MATERIALS AND METHODS

For this study, spores of *Equisetum arvense* L. were collected on the left bank of the Tisza River at Szeged by I. Gáspár on 5 April, 1992. Spores were irradiated with a CuKα beam (35KV, 20mA) for 5s, 15s, 30s or 60s using a BRON-OM1 "apparatus" in the Radiological Laborotury, Department of Mineralogy, Petrology and Geochemistry, J.A. University, Szeged. After irradiation, the spores were fixed in Millonig buffered 1% osmium tetroxide for l hour, after which they were washed in an osmium free, 0.2M phosphate buffer overnight. Specimens were dehydrated in an ascending series of ethanol, including uranyl acetate staining in 70% ethanol, in 15 min steps. Samples were embedded in Durcupan (Fluka) araldite epoxy resin in gelatin capsules and polymerized in a thermostat at 56℃ for 2 or 3 days. Ultrathin sections were cut on a Porter Blum ultramicrotome in the Electron Microscopical Laboratory of the Institute of Biophysics, Biological Center, Hungarian

Academy of Sciences. Transmission electron micrographs were taken using a Tesla BS-540 (resolution 6-7 Å) and a Zeiss EM-902 (resolution 2-3 Å). Nomenclature for the different layers of the spore wall followed Tryon and Lugardon (1991).

RESULTS

Experiment: 1726 (Plate l, figs. 1-4)

The general survey picture of the spore (Plate l, fig. 1), illustrates well the different layers of the wall and a part of the protoplasm. On the perispore the small, granular, superficial elements are not so discernible. This is well shown in picture 2 in Plate l. Tiny electron dense granules are on the outer and inner surface of the perispore and the "pseudo-orbicules" are damaged. After irradiation, appears to be the exospore three layers (a-c) with the outer and the inner is more electron dense than the middle. (Plate l, fig. 1). There are small electron dense granules on the surface of the exospore (Plate 1, figs. 2, 3). The endospore (Plate 1, fig. 3) was damaged, in some part a finely granular ultrastructure may be discernible. The plasma membrane and the protoplasm were severely damaged (Plate l, fig. 3). Structures in molecular dimension are well shown in a highly magnified picture at high resolution (Plate 1, fig. 4).

Experiment: 1727 (Plate 2, figs. 1,2)

Picture 1 of Plate 2 illustrate well the general characteristics. Several micro-organisms are on the surface of the perispore, some are attached to a globular unit on the surface (pseudo-orbiculum). Micro-organisms were observed in the hole of the perispore and exospore (Plate 2, fig. 2). The granular superficial elements were present in this treatment. Small electron dense globular particles were not observed on the surface of the perispore. The inner layer of the exospore (C) is well separated from the middle layer by its stronger electron density. The outermost layer is not so characteristic.

Experiment: 1728 (Plate 2, figs. 3, 4)

Degradation process was observed on the surface of the perispore (Plate 2, fig. 3). Small globular units were observed on the surface in the macromolecular dimension. Secondary lamellar structures appeared on the inner surface in all probability. The exospore (Plate 2, fig. 4) was little damaged, fine granular structures appeared.

Experiment: 1729 (Plate 3, figs.1-4)

This was the strongest X-ray dose to this spores. The degradation of the outer surface of the perispore is very typical, (Plate 3, fig. 2) particularly at some of the superficial globular units. The electron dense particles were in the dimension of the larger molecules. Some not so clear regular pentagons are also observed. In the inner part of the perispore in some places the electron dense globular units are aligned. The inner, lamellar system is in some places perceptible. The three layers (A-C) of the exospore are characteristic (Plate 3, fig. 1,4). There are tiny electron dense granules on the surface of the exospore (Plate 3, fig. 1,4). At the exospore endospore border one lamella is well shown in picture 1 in Plate 3. The plasma membrane and, in general, the protoplasm disintegrated following X-ray irradiation (Plate 3, figs. 1, 3).

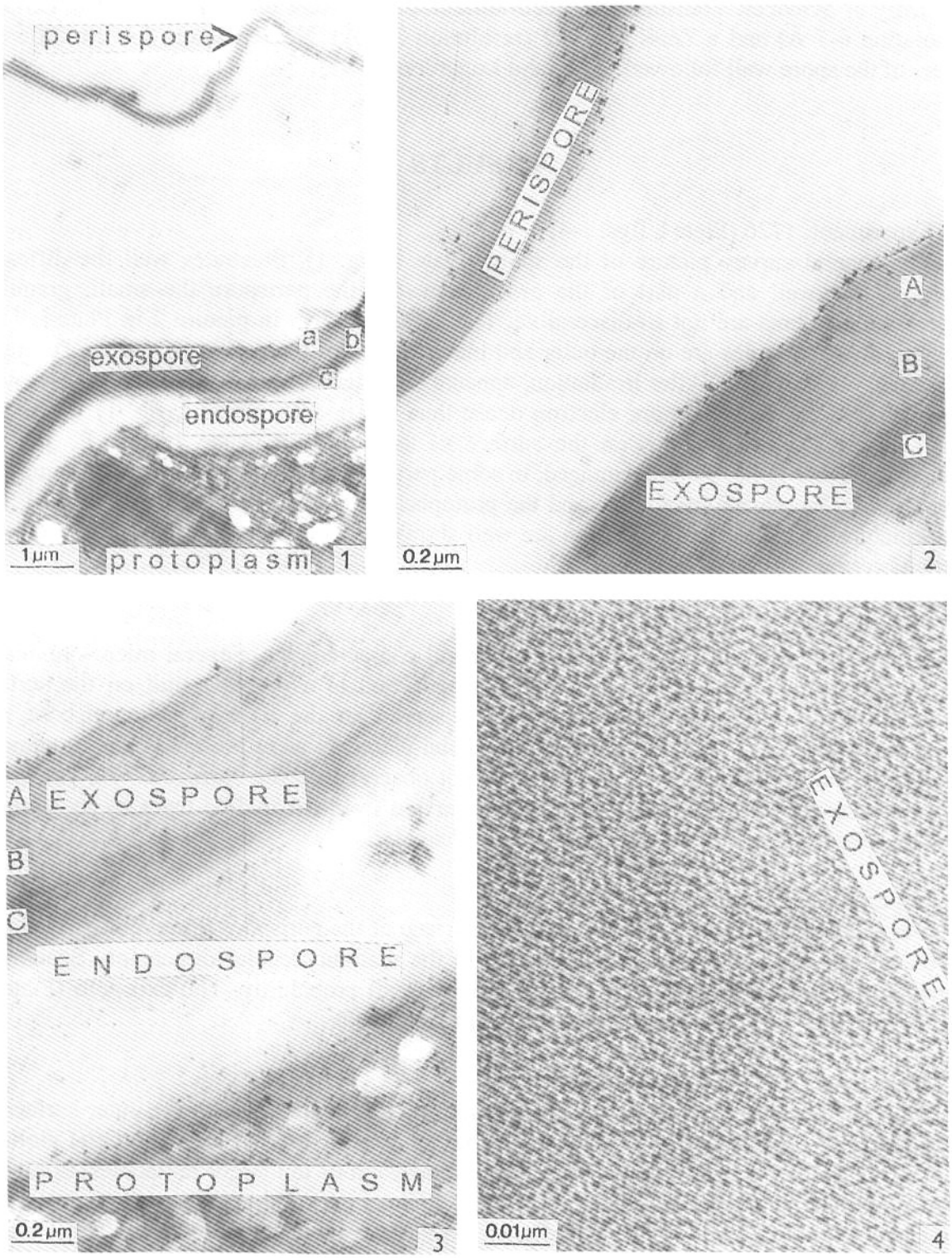


Plate 1. Figs. 1-4. *Equisetum arvense* L. TEM of X-ray irradiated spores. Experiment No: 1726. Fig. 1: General survey picture of wall stratification. Negative no: 6139, x 10,000. Fig. 2: Highly magnified picture of the X-ray irradiated perispore and exospore. Negative no: 6145, x 50,000. Fig. 3: Detail of the fine structure of the exospore, endospore and the protoplasm. Negative no: 6141, x 50,000. Fig. 4: Biopolymer system of an X-ray irradiated exospore. Negative no: 5005, x 1,000,000. A-C, three layers of the exospore.

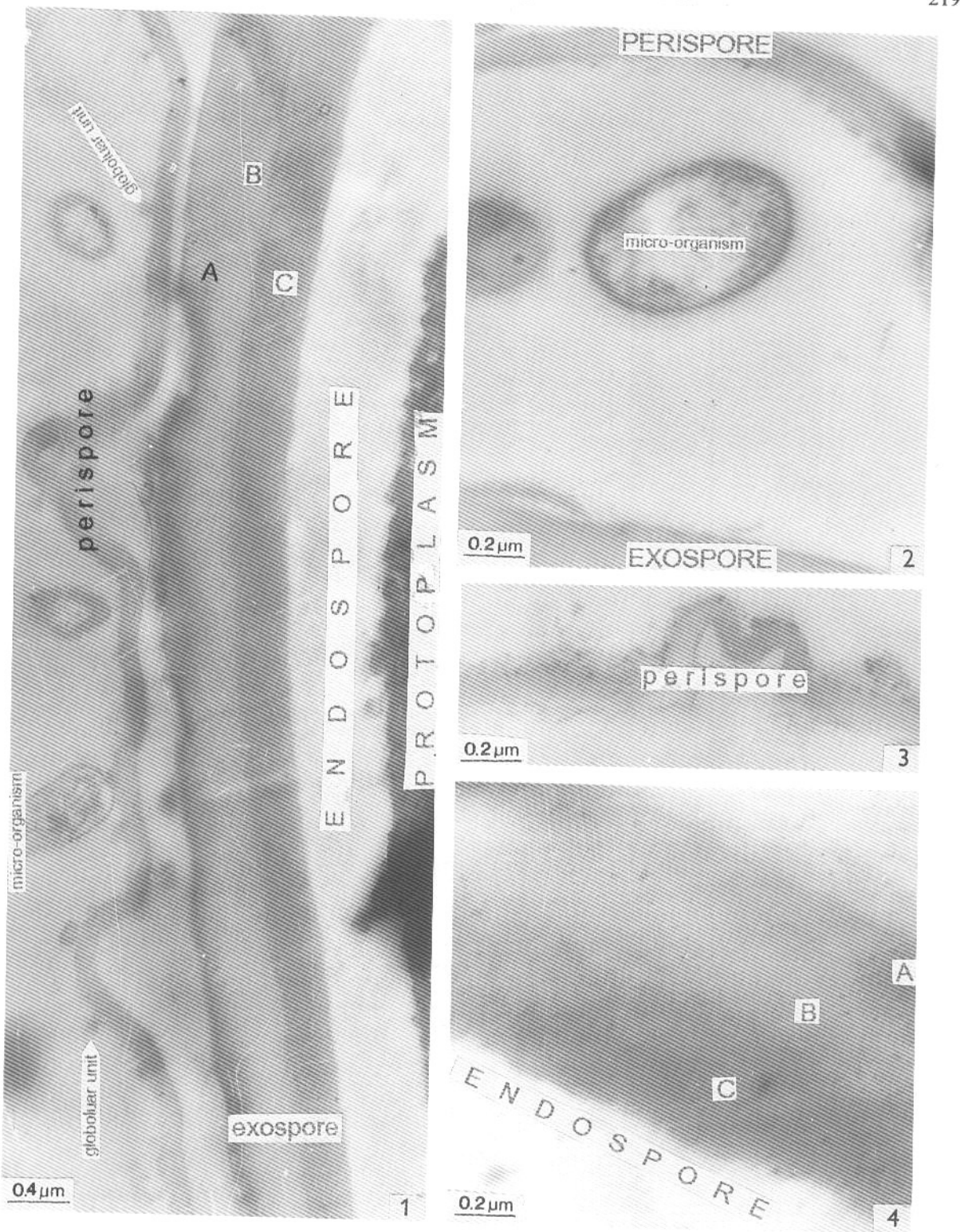
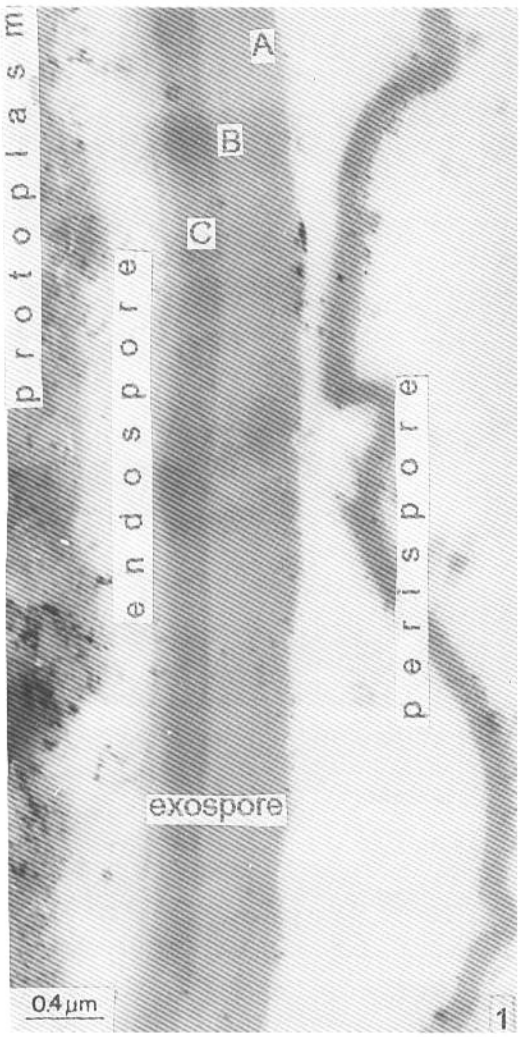
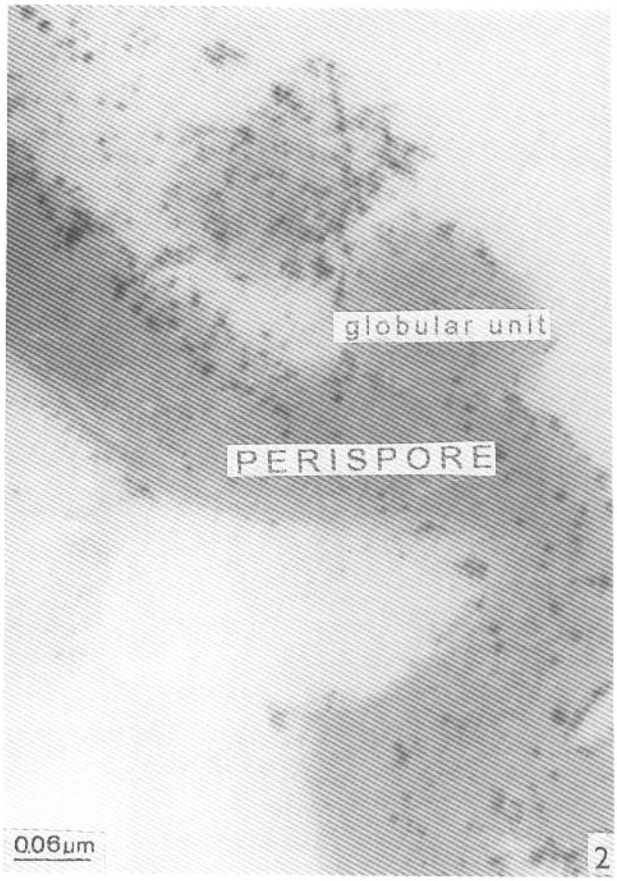
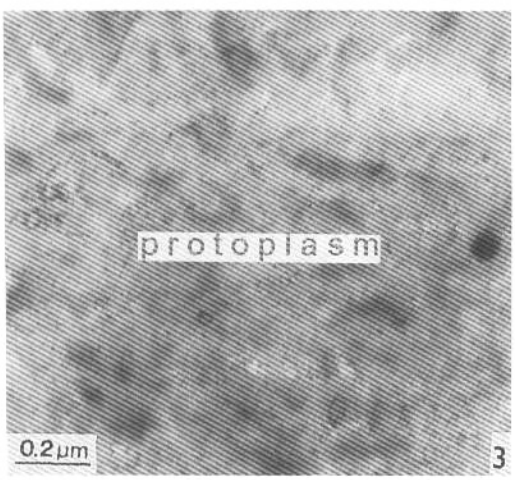


Plate 2. Figs. 1-3. *Equisetum arvense* L, TEM of X-ray irradiated spores. Experiment No: 172. Fig. 1: General survey picture of the fine structure of the spore. Illustrated are the globular units of the perispore and different kinds of micro-organisms of the perispore. Negative no: 6154, x 25,000. Fig. 2: Micro-organism within the space between the exospore and the perispore. Negative no: 6154, x 25,000. Fig. 3: Detail of the fine structure of the perispore. Negative no: 6168, x 50,000. Fig. 4: Experiment No: 1728. Detail of the ultrastructure of the X-ray irradiated exospore. Negative no: 6170, x 50,000. A-C, three layers of the exospore.







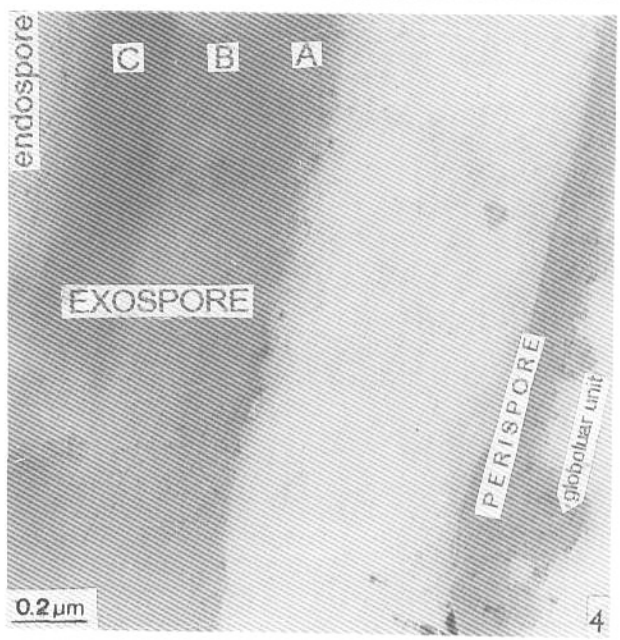


Plate 3. Figs. 1-4. *Equisetum arvense* L. TEM of X-ray irradiated spores. Experiment No: 1729. Fig. 1: General survey picture of the ultrastructure of the spore. Negative no: 6178, x 25,000. Fig. 2: Highly magnified picture of the perispore. Well shown are the degraded superficial globular units. The dark globules are in the macromolecular dimension. Negative no: 4993, x 150,000. Fig. 3: Ultrastructure of the degraded protoplasm: the fine structure of the thylakoid membrane system are not discernible. Negative no: 6176, x 50,000. Fig. 4: Detail of the ultrastructure of the exospore and the perispore. Negative no: 6180, x 50,000. A-C, three layers of the exospore.

DISCUSSION

In comparison to the TEM results of the non-experimental (fresh) spores, the following can be pointed out:

1. The ultrastructure of the exospore was also the subject of several papers based on the differencies in the electron density. Lugardon (1975), Nilsson *et al.* (1977) described the exospore as two layered. Uehara and Kurita (1989) established also two layers by the electron density on the exospore, but in the earlier stage the outer layer is darker than the inner one. Nilsson *et al.* (1977) described small electron dense (darkly stained) granules on the surface. Beneath the exospore, characteristic lamellar ultrastructure was described. In consequence of the X-ray irradiation the degradation of the perispore was well shown in particular after the strongest dose. A macromolecular system may be investigated by this method. After irradiation the exospore is three layered, and it is worth of mentioning that more or less the inner layer is more electron dense. The endospore is thickened secondarily, and degraded.
2. Molecular system may be discovered by irradiation of the exospore.
3. The plasma membrane and the protoplasm degraded after the lowest X-ray dose.
4. Finally, it is interesting to note that the thick exospore of the *Equisetum arvense* may be dissolved very easily by diethylamine (cf. Kedves and Gáspár, 1994a, b) while the other layers of perispore and the elaters are resistant.

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問荊孢子經 X 射線照射後之微細構造研究[1]

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摘　　要

以 X 射線照射問荊(*Equisetum arvense* L.) 之孢子各 5 秒、15 秒、30 秒或 60 秒後，利用穿透式電子顯微鏡觀察其孢子壁與原生質微細構造之變化。結果發現，問荊之外孢子對於 X 射線的抗力較對二乙胺有機溶劑的抗力大，而孢子壁的分子結構亦可藉由 X 射線照射顯現出來。由本研究可知，孢子壁各層次對於放射線及有機溶劑的抗性表現不同，故可藉此瞭解孢粉質的生物多聚體系統構造。

關鍵詞：問荊孢子，微細構造，X 射線效應。

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