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IN VITRO DESTRUCTION OF THE EXINE OF RECENT PALYNOMORPHS I

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Abstract

During our investigations on recent and fossil pollen grains and other plant microfossils (e.g. *Botryococcus braunii* KÜTZ., extracted from oil shale), the wall structure was degraded enzymatically under in vitro experimental conditions. On the basis of our first results we have established the following: 1. *Helix* enzyme with merkapto-ethanol is suitable to decompose the sporopollenin of recent and fossil plant microfossils. 2. The partially decomposed wall, studied by the TEM method, may reveal the molecular structure of the sporopollenin. 3. Our results on recent *Corylus avellana* L. pollen grains suggest a globular structure of the biopolymers of the sporopollenin of this species.
Key words: Corylus avellana, sporopollenin, molecular structure

Introduction

„Sporopollenins are probably the most resistant organic materials of direct biological origin found in nature and in geological samples" (BROOKS and SHAW, 1978, p.91). The chemistry of this material has been the subject of several studies. The first results were published by JOHN (1814) and BRACONNOT (1829). ZETZSCHE et al. (1931), ZETZSCHE and KÄLIN (1928), KWIATKOWSKI and LUBLINER-MIANOWSKA (1957), and MÄDER (1958) published further data. These first results were rewieved by TOMSOVIC (1960) and it was emphasized that sporopollenin is a high-polymerized terpene derivate, similar to cutin. The results of SHAW and YEADON (1964) and BROOKS and SHAW (1968a,b) fundamentally changed this concept, and the importance of carotenoids was emphasized in the composition of sporopollenin. SHAW (1971) wrote as follows; p.305: „sporopollenins are oxidative polymers of carotenoids and/or carotenoid esters". As regards the enzymatic degradation of the exine, the publications of ELSIK (1966, 1971) are worth mentioning. In 1971 he emphasized the following: „Microbial degradation of sporopollenin which results in definite patterns or scars is attributable to higher bacteria (*Actinomycetes*) and true fungi". Biological (enzymatical) destruction as a method in the research of the molecular structure of sporopollenin was not yet used. The first observations of microbial destruction of the pollen wall were published by ERDTMAN (1971). During the ontogeny of the pollen wall HORVATH (1969) and FLYNN and ROWLEY (1971) demonstrated acid phosphatase reaction. FAEGRI (1971) p.261 wrote: „Sporopollenin may be no exception, to enzymatic degradation, but at the moment we do not know

very much about these enzymes which are able to digest this material. I was charmed by the idea presented at the Symposium by HESLOP-HARRISON that the final stages in the formation of sporopollenin may be a non-enzymatic reaction, which suggests that there are no enzymes able to reverse the process if the chemical equilibrum is disturbed." KEDVES et al. (1974) recognized the molecular structure of degraded exines from Eocene of Mississippi, USA. A globular structure of the sporopollenin was established, moreover it was established, that „new studies and experiments will be carried out on this subject" (p. 436). SENGUPTA and ROWLEY (1974) and ROWLEY (1975, 1978) published the concept that the exine consists of filamentous subunits (cf. ROWLEY 1962a, b; ROWLEY, DAHL, SENGUPTA and ROWLEY, 1981). The motto of this last mentioned paper is greatly appreciated; „Between 'yes' and 'no' there are possible answers, less abrupt more fruitful" – HEISENBERG – .

Taking into consideration the above mentioned results and different concepts, we carried out our experimental studies on recent and fossil palynomorphs. This paper summarizes the first our results in this field.

Material and Methods

Fresh pollen grains of *Corylus avellana* L. were collected on 11 March in 1984 in the Botanical Garden of the J.A. University by I. GYURICZA and I. DÁVID. The pollen material was placed into dark glass containers to avoid autoxidation of the sporopollenin. The first experiments were made on 17 April 1984. The experimental methods are essentially those employed for the preparation of protoplasts for further experimental studies (DAVIS, 1985, PEBERDY, 1985). We tried the following procedures:

C–1 – 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, temperature 30 °C, length of time: $2^h30'$.
C–1A – the same only the lenght of time was 5^h.
C–2 – 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, + 1 ml merkapto-ethanol, temperature 30 °C, length of time : $2^h30'$.
C–2A – the same only the length of time was 5^h.
C–3 – 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, + 1 ml merkapto-ethanol + 20 mg. EDTA, temperature 30 °C, lenght of time: $2^h30'$.
C–3A – the same only the length of time was 5^h.

The procedures of the second experiments (15th May, 1984) were as follows:

C–4 – 20 mg. air dried pollen grains + 20 ml H_2O dest., temperature 30 °C, length of time: $2^h30'$.
C–4A – the same only the length of time was 5^h.
C–6 – the same as C–1.
C–6A – the same as C–1A.
C–8 – 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, + 20 µl merkapto-ethanol, temperature 30 °C, length of time: $2^h30'$.
C–8A – the same only the length of time was 5^h.
C–10 – 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, + 20 µl merkapto-ethanol + 20 mg. EDTA, temperature 30 °C, length of time: $2^h30'$.
C–10A – the same only the length of time was 5^h.

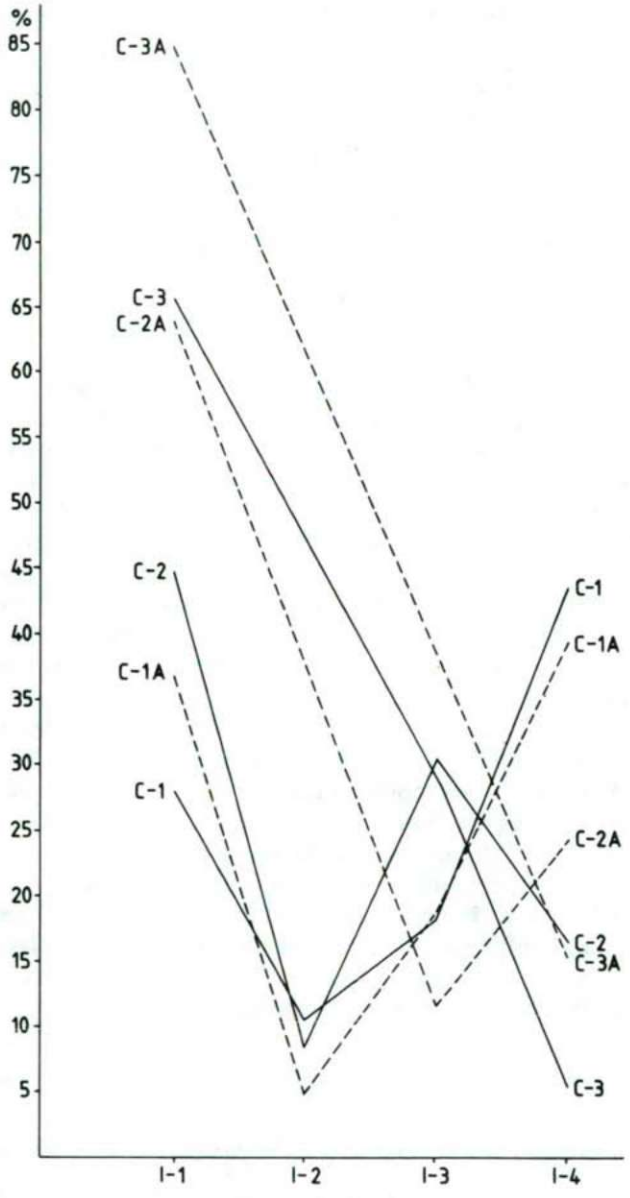

Fig. 1

The percentage of different forms of *Corylus avellana* L. pollen grains; experiments: C–1, C–1A, C–2, C–2A, C–3, C–3A. I–1 = empty, typical pollen grains, I–2 = pollen grains which are full of protoplasm, with small oncus, I–3 = pollen grains with normal size range onci, I–4 = pollen grains full of protoplasm, without oncus, pro parte near protoplast.

The procedures of the third experiments (26th September, 1984) were as follows:

C–4c.1 – 20 mg air dried pollen grains + 1 ml merkapto-ethanol + 2 ml H_2O dest., temperature 30 °C, length of time: $2^h30'$.
C–4c.2 – the same only the length of time was 5^h.

The pollen material was fixed on OsO_4 (aqu. dil.) and embedded in Araldite (Durcupan, Fluka). For LM investigations, pollen grains in Araldite were mounted on slides. The ultra-thin sections were made on a Porter Blum ultramicrotome with glass knives. The TEM photomicrographs were taken on a TESLA BS–500 instrument, which has a resolution of 6 Å.

Results

1.The most important changes of pollen grains as a result of enzymatic activity were followed by the LM method (Plate I, fig.1–4). Several types and forms, apart from the basic form of *Corylus* pollen, which is triaperturate with a triangular equatorial outline, may be distinguished. From the point of view of our research the „near protoplast" is important (Plate I, fig.4). The exine of this kind of pollen grain is very thin, without stratification, lacks the apertural exine, and the form is globular. To prepare this pollen form, procedures no. C–2, and C–2A were succesful. The quantitative proportions of the different forms, following the three series of experiments are figured on fig. 1–3, but the last type represents all pollen grains which are full of protoplasma. Fig. 4 represents the per cents of the „near protoplast" sensu stricto, and all other pollen grains, which are full of protoplasm. A regularity may be established, and on the basis of the present results the middle values are important in respect of the investigation of the molecular structure of the sporopollenin. Moreover a protoplasma/enzyme interaction may be presumed to occur during the exine degradation.

2. On the basis of the TEM data we may establish the following:

C–1 (Plate I, fig. 5) In the ultrastructure of the pollen grains, there is no obvious destruction. The exine is as has been described in earlier papers (channeled tectum, irregular or granular infratectum, no endexine under the foot layer except the apertural area). In the protoplasm the small oncus in the apertural region is characteristic and the finely lamellar intine is thin.

Plate I

LM and TEM pictures from pollen grains of *Corylus avellana* L. experimented upon.

1–4. Light microscopic pictures of the different pollen forms of *Corylus avellana* L. x1000.
1. Empty pollen grain, typical form; C–1.
2. Pollen grain full of protoplasm, with small oncus, typical form; C–1.
3. Onci are within normal size range; C–1A.
4. Almost naked protoplast, lacks the apertural exine, and the pollen wall is extremely thin, degraded; C–2.
5. TEM picture. The non-degraded ectexine, the oncus (0), and the protoplast are shown; C–1, x5000.


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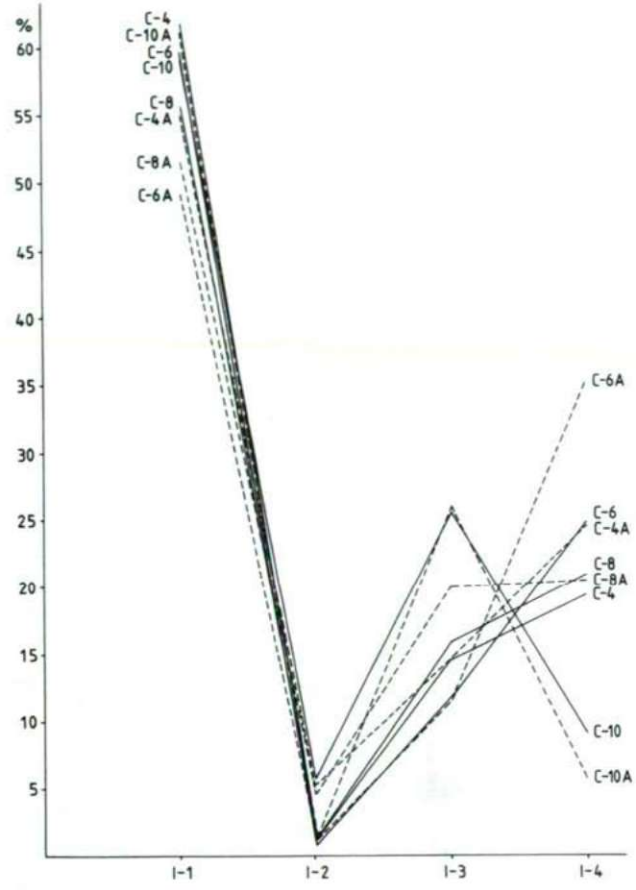


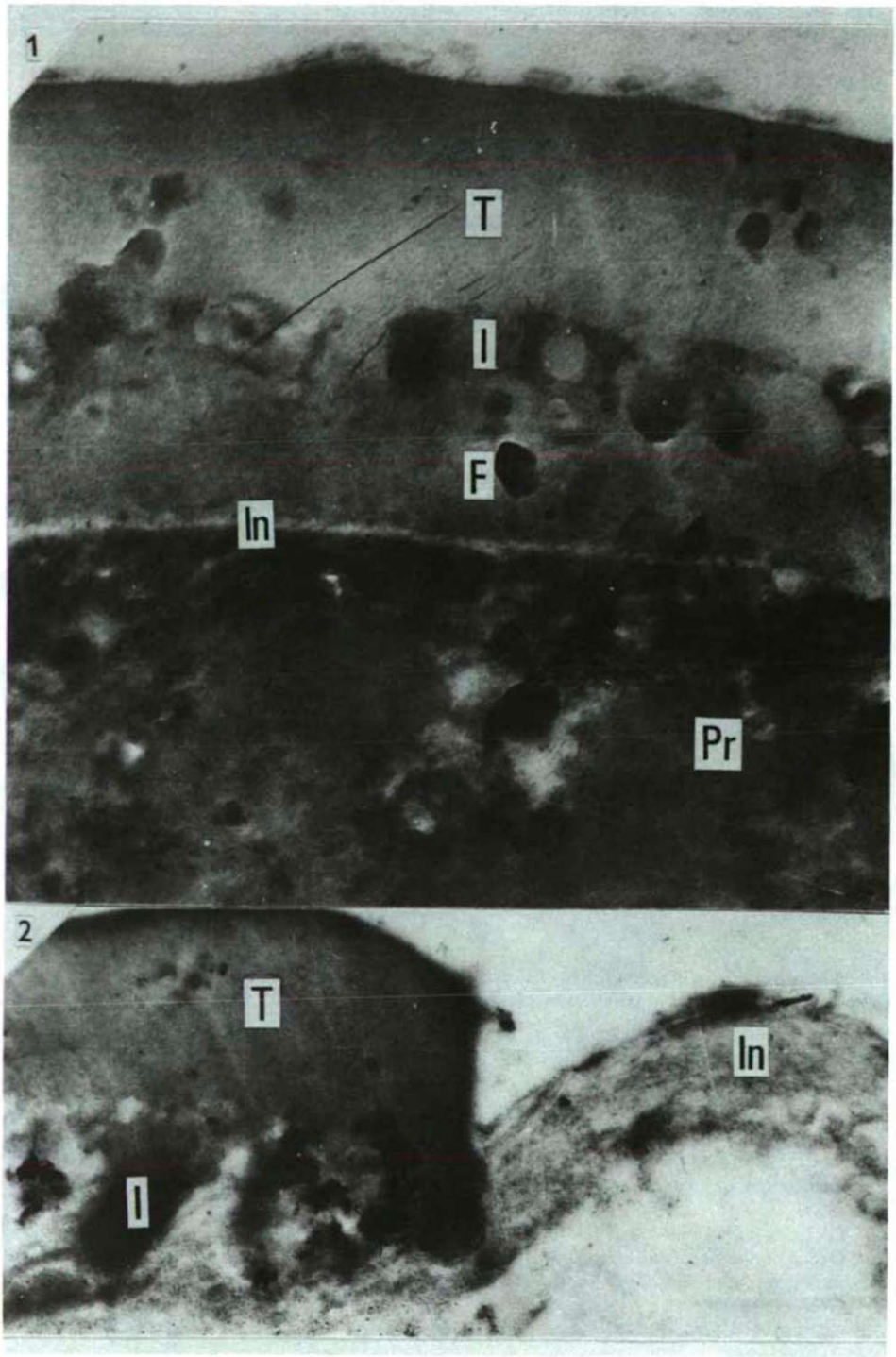
Fig. 2

The percentage of different forms of *Corylus avellana* L. pollen grains; experiments: C–4, C–4A, C–6, C–6A, C–8, C–8A, C–10, C–10A. I–1 = empty, typical pollen grains, I–2 = pollen grains which are full of protoplasm, with small oncus, I–3 = plasmolysed pollen grains, I–4 = pollen grains full of protoplasm, without oncus, pro parte near protoplast.

Plate II

TEM pictures.

1. Detail from the inter-apertural exine, including: the tectum (T), the infratectal layer (I), the foot layer (F), the very thin intine (In), and the protoplasm (Pr). The degradation of the infratectal layer is shown, channels were not observed in the tectum; C–1A, x100000.
2. Ultrastructure of the apertural intine (In). The lamellar ultrastructure is shown; C–2, x50000.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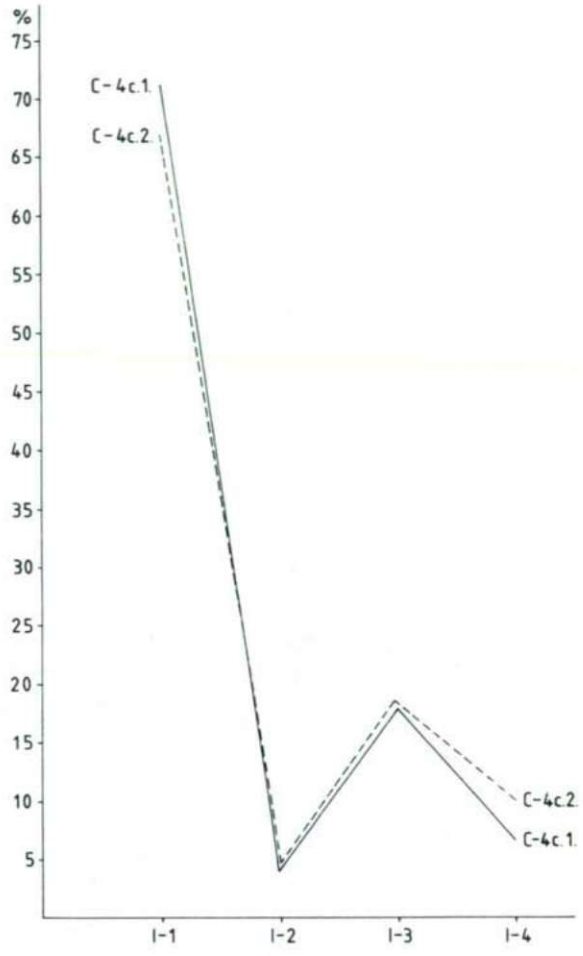
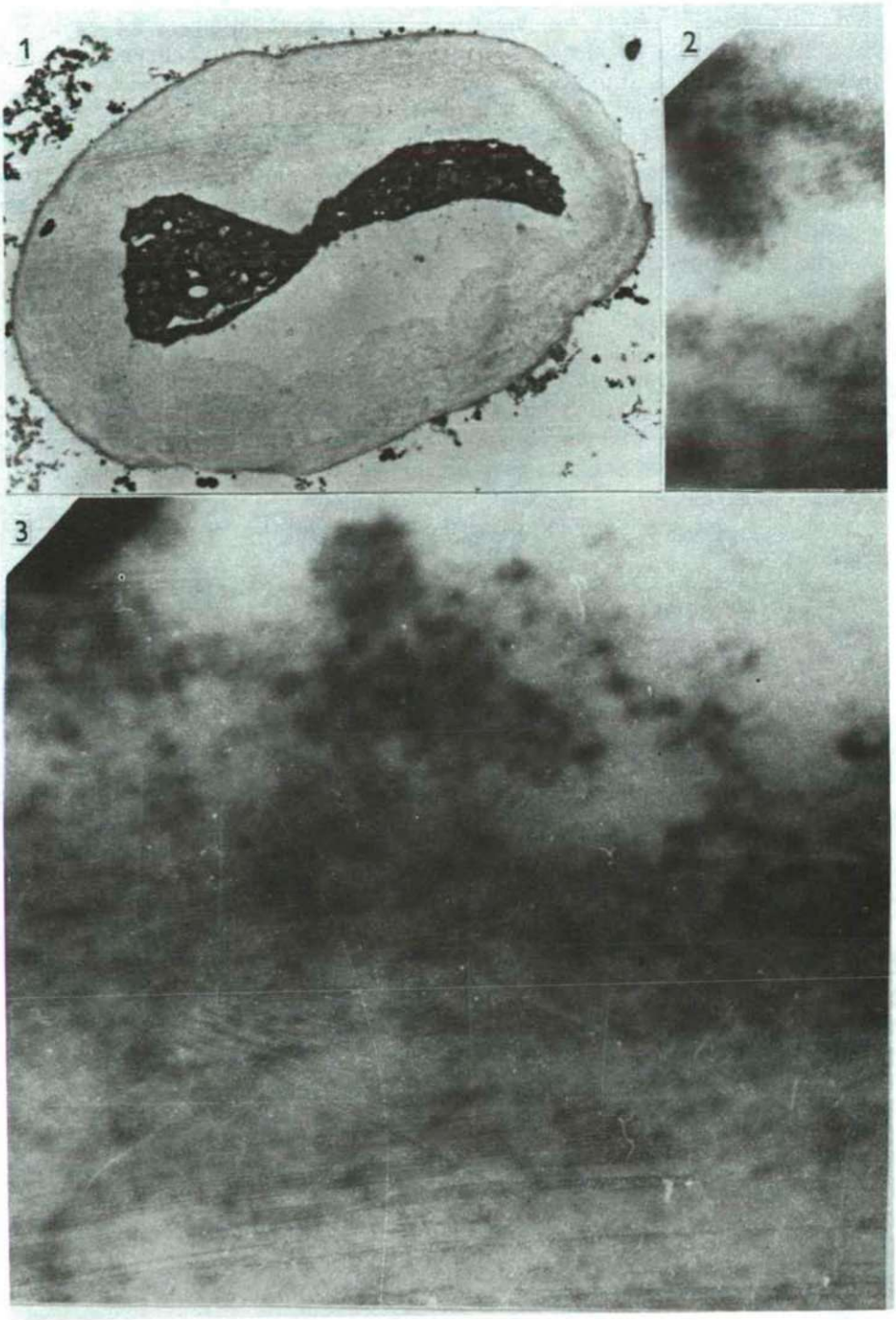


Fig. 3

The percentage of different forms of *Corylus avellana* L. pollen grains; experiments: C–4c1, C–4c–2. I–1 = empty, typical pollen grains, I–2 = pollen grains which are full of protoplasm, with small oncus, I–3 = plasmolysed pollen grains, I–4 = pollen grains full of protoplasm, without oncus, pro parte near protoplast.

Plate III

1. Ultrastructure of an almost naked protoplast of *Corylus* pollen, showing the remainder of the ectexine, and the lack of the apertural area. There is a lamellar ultrastructure is present under the ectexine remains, similar to the intine. The degradation of the protoplasm is also shown; C–2A, x5000.
2. Globular units of sporopollenin from the specimen illustrated on fig.1; C–2A, x100000.
3. Detail of the above mentioned enzyme degraded ectexine. Illustrated are the remnants of the infratectum and the foot layer, the globular sporopollenin units of the ectexine are well shown; C–2A, x500000.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C–1A (Plate II, fig.1) An interesting partial degradation of the exine was observed firstly on the infratectal layer. It is also interesting, that channels were not observed on these preparations. During the ontogenesis of the ectexine, in the first stage of its development, the probacules appear, e.g. the infratectal layer, and afterwards the tectum.

C–2 The above mentioned partial degradation is more definite. The apertural area lacks the inner, lamellar endexine, but the pore-covering lamellar intine is not degraded (Plate II, fig.2).

The most important results were obtained by experiment C–2A. On microphotograph 1 of Plate III, two important things may be observed: 1. The ectexine degradation is the final stage; it is very thin and without stratification. 2.The degradation of the protoplasm is well shown and a relatively thick layer with lamellar ultrastructure is present, similar to the intine. Several pictures were taken with high magnification of this degraded part of the exine, and in this way globular units were observed of 8–13 Å in diameter (Plate III, fig. 2,3).

C–3 and C–3A experiments gave heterogeneous results, it seems, that EDTA is not advantageous for the degradation of sporopollenin.

The most important results of the second and third series of experiments are as follows:

C–4 and C–4A — No alterations in the fine structure of the exine.

C–8A and C–10 — Partial degradation of the ectexine was observed, in particular in the infratectum, and the channels of the tectum were also not observed; cf. C–1A.

C–10A — Globular units were observed in several thin sections. Worth of mentioning is, that the merkapto-ethanol without *Helix* enzyme have degraded only in insignificant degree the exine of the *Corylus* pollen. The channels of the tectum were not observed, and the degradation of the endexine in the apertural region was also observed.

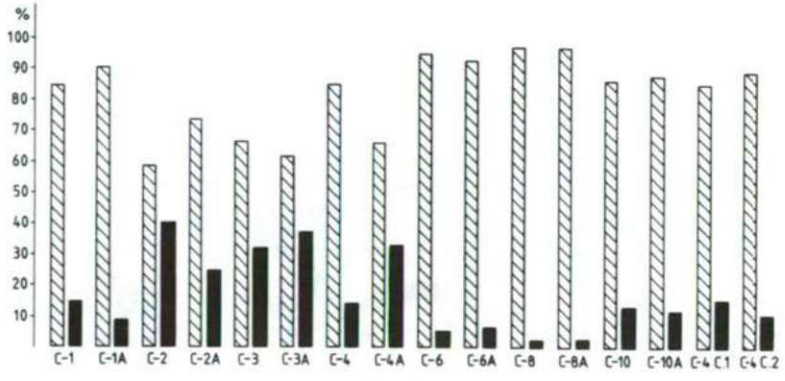

Fig. 4

The per cents of the almost naked protoplast — black column -, and all other pollen grains, which are full of protoplasm; streaked column.

Discussion and conclusions

1. As new result we emphasize that *Helix* enzyme with merkapto-ethanol is suitable for the destruction of the ectexine. In this way, combined with the TEM method, the molecular structure of sporopollenin may be demonstrated. Taking into consideration the digestion of *Helix pomatia* it may be presumed that this method will be useful in the research of the molecular structure of all kinds of plant cell walls.

2. We found globular units for the polymers of sporopollenin, but non-granular units are well shown on several pictures of ROWLEY, DAHL and ROWLEY (1980,1981), and of ROWLEY (1981). But on the other hand, in several aspects their results are similar to ours, e.g.: ROWLEY (1967), ROWLEY, DAHL, SENGUPTA and ROWLEY (1981). Probably the basic elements are globular, and these elements may be arranged into units of higher order; filaments, helicoide structures, etc.

3. 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.

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