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# THE MICROSPORIDIAN MUCOCALYX AS SEEN IN THE SCANNING ELECTRON MICROSCOPE

J. VÁVRA and R. J. BARKER

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, and Imperial College Field Station, Ascot

**Abstract.** The mucous layer (mucocalyx) surrounding the spores of *Gurleya elegans* can be revealed in the scanning electron microscope only when freeze drying or critical point drying is used during spore material preparation. Best preservation is obtained after critical point drying when the mucocalyx appears as a halo of fine fibres forming a dense anastomosing mesh around each spore. Scanning electron microscope demonstrates clearly the bulk of the mucocalyx material around each spore and supports thus the assumption that the mucocalyx is a flotation device.

Spores of many aquatic microsporidia are surrounded by a thick layer of material impermeable to particles of India ink (Lom and Vávra 1961, 1963, Weiser 1963). This material was originally called "mucus" but later the term "mucocalyx" was proposed (Vávra and Sprague 1976).

The mucocalyx is thought to be a flotation device but no attempt to prove this function experimentally has been made.

The presence or absence of the mucocalyx is an important feature in microsporidian taxonomy. This is the reason for the present investigation of the mucocalyx with the scanning electron microscope, an instrument finding an increasing use in microsporidiology. Another reason for this investigation is the necessity to compare the structure of the mucocalyx as revealed in the scanning electron microscope with data on the mucocalyx fine structure previously obtained from the transmission electron microscope (Vávra 1968).

## MATERIAL AND METHODS

Spores of the microsporidian *Gurleya elegans* were selected for our investigation. In this species the mucocalyx is developed as an important layer of material completely surrounding the spore and with a thickness exceeding the spore length (Komárek and Vávra 1968) (Plate I, Fig. 2, inset). The spores were obtained from infected copepods *Cyclops strenuus* collected during the winter of 1976/77 in a small pond in the village of Šeberov near Prague. The infected females of the copepod were triturated in a glass homogenizer and the spores were cleaned from debris by repeated centrifugation. The spores were mounted on coverslips by a method described below. They were examined either fresh or fixed in a hanging drop with osmium vapours. Three alternative methods were used for the drying of the spores prior to observation in the electron microscope: simple drying in air (AD) of a drop of spores suspended in distilled water, freeze drying of a similar suspension of spores (FD), critical point drying (CPD). For the freeze drying a minute droplet of spores suspended in water was frozen at liquid nitrogen temperature and was dried "in vacuo" under continuous cooling at —70 °C. The critical point drying was performed on spores dehydrated in a graduated series of ethanol, transferred into amyl acetate and dried from carbon dioxide. After drying the spores were vacuum coated with a thin layer of carbon and gold. A JEOL JEM 100B/ASID scanning electron microscope was used for observations.

A special method of mounting the spores was used in order to eliminate the loss of material experienced when the spores were directly applied to stubs before critical point drying. In this method the spores were first applied in a water suspension to a small round coverslip of a size that fitted the microscope stub. Most of the water was allowed to evaporate but full drying was carefully avoided.

The whole surface of the coverslip with spores was then covered by thin formvar film obtained in the same way as that used for coating electron microscope grids. The formvar film was applied to the coverslip with a wire loop. The membrane adheres firmly to the non-wet portion of the coverslip and effectively protects the spores from floating away during ethanol dehydration. The formvar film dissolves when the preparation is transferred to amyl acetate but at that time the spores adhere more strongly to the coverslip, being fixed to its surface by the coagulation of the spore surface under the influence of ethanol.

## RESULTS

The spores which were air dried, either fresh or in a fixed state, were collapsed, especially in the anterior region. Sometimes a deep artificial invagination was also present near the posterior end of the spore (Plate I, Fig. 1). The surface of such dried spores was nearly smooth, showing only a very fine and irregular granularity (Plate I, Fig. 2). There was no indication of the presence of the mucous layer on the surface of the air dried spores. Sometimes a strand of ill-defined material stretching from the posterior region and connecting the spores originating from a single pansporoblast was revealed (Plate I, Figs. 1, 2). Very small strands of such material were sometimes present also at the anterior pole of the spore (Plate I, Fig. 2).

After freeze-drying the preservation of the spore shape was generally better, but in many spores the mucocalyx was reduced to a few strands of material at the spore pole (Plate I, Fig. 3). However, in some other spores subjected to freeze-drying, the mucocalyx was revealed in the form of a thick layer of anastomosing fibres of irregular thickness. In many places the fibres appeared "melted" together into sheets of irregularly granulated material (Plate I, Fig. 4).

After critical point drying the spores were completely surrounded and obscured by a halo of fine fibres forming a dense and anastomosing mesh stretching out from the spore surface for a considerable distance (Plate II, Fig. 5). There was a difference in the appearance of this fibrillar material in the fresh and the fixed specimens. In the latter material the fibres were thin and formed a loose web around the spore (Plate II, Fig. 6). In the unfixed spores the fibres were much thicker, shorter and showed a tendency to collapse and to "melt" into a coarsely granulated material (Plate II, Fig. 7).

## DISCUSSION

Our observations show that the microsporidian mucocalyx can be successfully revealed in the SEM provided that suitable technique for the drying of the spores is used. The complete absence of the mucocalyx layer in the AD spores on one hand and the fair preservation of the same mucocalyx in both the FD and CPD spores on the other hand, is striking. This clearly shows that conclusions formed on the presence of the mucous layer around the spore in a simply dried material are unreliable. The same might be true for other types of spore surface structures. This is corroborated by our experience with *Tuzetia debaisieuxi* (formerly called either *Pleistophora debaisieuxi* or *Microsporidium debaisieuxi*). In this species a system of tubules attached to the surface of the spore is demonstrated by transmission electron microscope (Vávra 1965). However, when the spores are observed in the SEM after a simple drying, they appear to be smooth. The tubular ornamentation of the exospore is demonstrated only after critical point drying of the spores (Vávra and Barker, unpublished).

It is thought that the mucocalyx layer on the surface of microsporidian spores is a flotation device. Although there is no experimental data supporting this assumption,

there are two reasons in its support. Firstly, the mucocalyx has so far been demonstrated only in microsporidia from aquatic hosts. Secondly, one species with an extensive mucocalyx—*Gurleya elegans*—can be readily demonstrated in the plankton of the ponds in which the infected copepods are abundant and is still found several weeks after it is no longer present in the copepod population (Komárek and Vávra 1968). The observation of the mucocalyx in the SEM demonstrates clearly its volume and importance. It seems also justified to suppose that the spores surrounded by such an important layer of material are more apt to be swallowed by the copepod host.

Finally, our observations confirm the fibrillar nature of the mucocalyx. The negative staining of the mucocalyx shows that its fibres are very thin, in range of 5—7 nm (Vávra 1968, 1976). One can hardly expect that such thin fibres could be readily demonstrated in the SEM. Apparently the fibres which we see in the microscope are bundles consisting of several finer fibres adhering together.

## ИЗУЧЕНИЕ МУКОЗНОГО СЛОЯ МИКРОСПОРИДИЙ ПОД СКАНИРУЮЩИМ МИКРОСКОПОМ

Й. Вавра и Р. Дж. Баркер

**Резюме.** Мукозный слой, окружающий споры *Gurleya elegans* можно изучать с помощью сканирующего электронного микроскопа только в том случае, если материал приготовлен с помощью высушивания на холоду или при критической точке. Более удобным является метод высушивания при критической точке, когда мукозный слой появляется как светящийся круг тонких волокон, образующих густую анастомозную сеть вокруг каждой споры. Сканирующий электронный микроскоп ясно показывает большое количество мукозного материала вокруг каждой споры и таким образом подтверждается предположение, что мукозный слой является устройством для плавания.

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J. V., Parasitologický ústav ČSAV, Flemingovo n. 2, 166 32 Praha 6, ČSSR

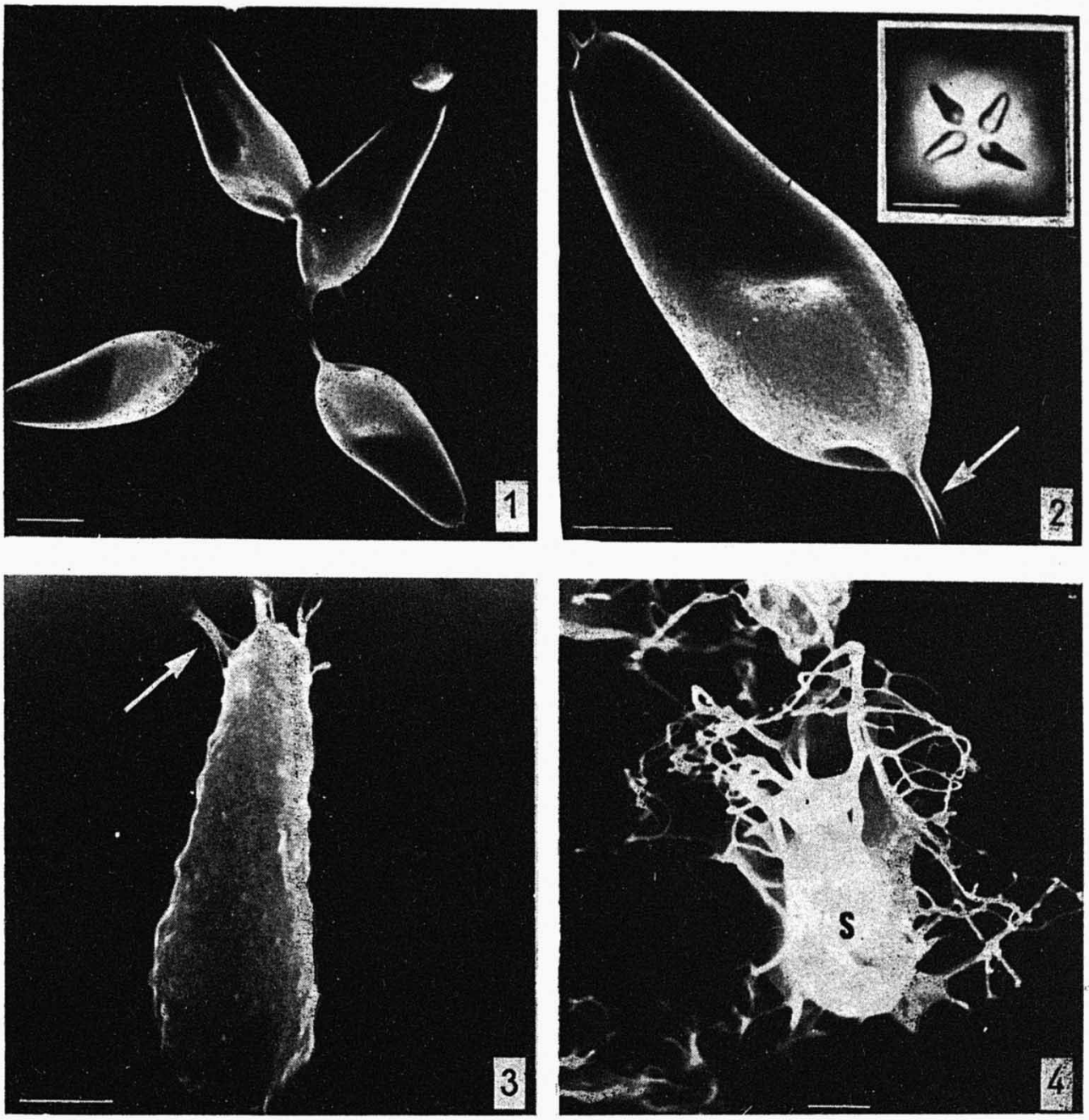
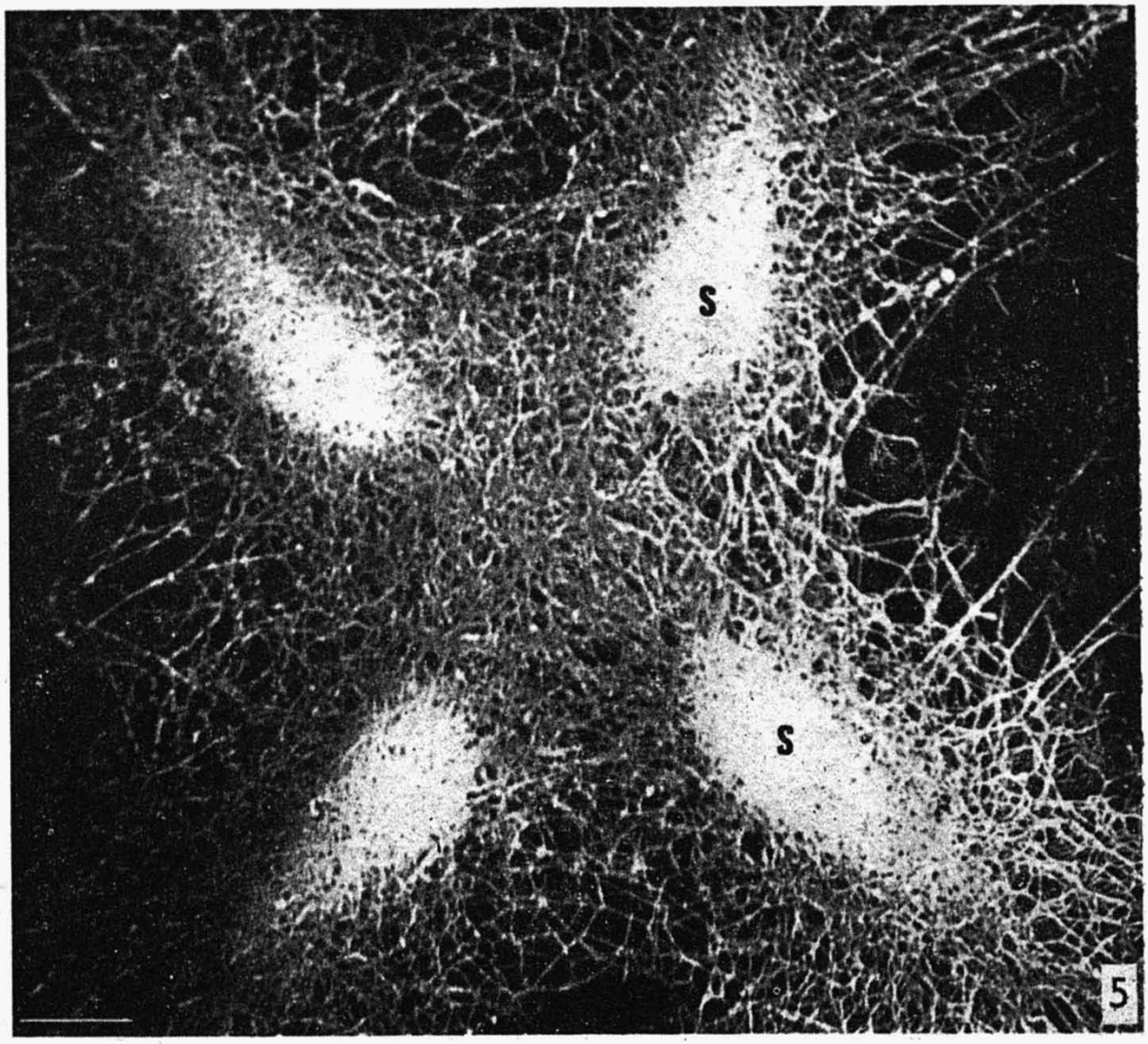
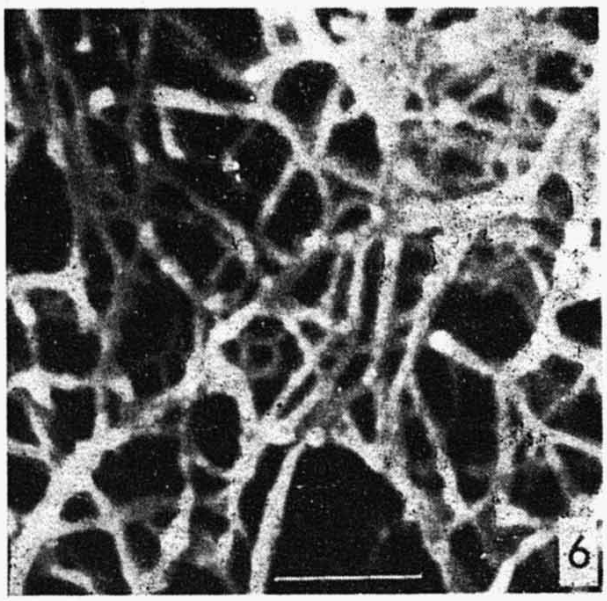



**Fig. 1.** *Gurleya elegans*, four spores of a pansporoblast in the scanning electron microscope. Air-drying, no fixation (Bar = 1 μm).
**Fig. 2.** Detail of Fig. 1 showing the spore collapse and the absence of the mucocalyx except of a few strands of ill defined material at the poles of the spore (arrow) (Bar = 0.5 μm). Inset: a pansporoblast of *G. elegans* in the India ink preparation observed under the light microscope (Bar = 10 μm).
**Fig. 3.** Spore of *G. elegans* in the SEM after freeze-drying of a non-fixed sample (Bar = 0.5 μm).
**Fig. 4.** Same sample as on Fig. 3 but an ill-defined mucocalyx is revealed around the spore (s) (Bar = = 0.5 μm).



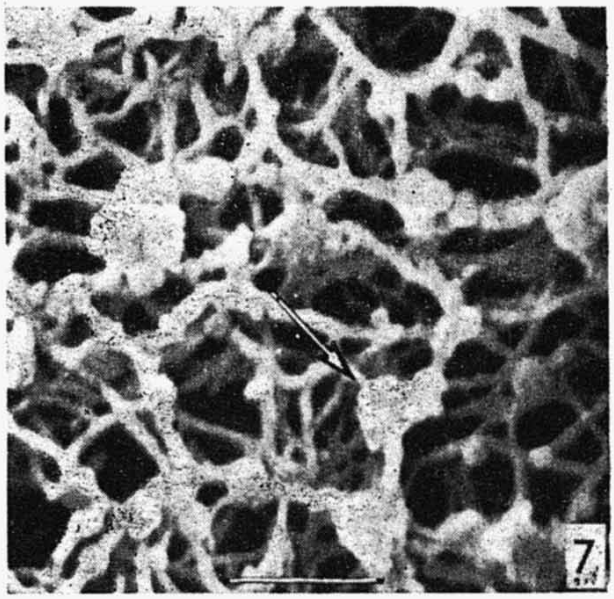




**Fig. 5.** Whole pansporoblat of *G. elegans* as seen in the SEM after osmium vapours fixation and critical point drying. The spores (s) are surrounded by the fibres of the mucocalyx (Bar = 0.5 μm). **Fig. 6.** Detailed view of the mucocalyx fibres after osmium vapours fixation and critical point drying (Bar = 0.2 μm). **Fig. 7.** Detailed view of the mucocalyx fibres on non-fixed spores after critical point drying. Individual fibres collapse artificially and melt together into granules (arrow) (Bar = 0.2 μm).