The Morphology of Solitary Adiaspores of *Emmonsia crescens* from the Lung of Man*)

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Abstract. At postmortem examination of the lungs of man occasional adiaspores were found resembling in size and appearance those of *Emmonsia crescens*. In three cases, mature adiaspores with a typically layered wall were present; in two of them an additional basophilic layer on the inner side was observed. In two cases the rather small adiaspores showed the pattern of differentiation. Tissue reaction was of different type.

Studies of coccidioidomycosis of small mammals in Arizona revealed mycotic pulmonary lesions caused by an unknown mycotic pathogen. The fungus was assigned to the genus *Haplosporangium* Thaxter under the name *Haplosporangium parvum* (EMMONS and ASHBURN 1942) and the term haplomycesis was proposed for this infection in the rodents. CIFERI and MONTEMARTINI (1959) excluded the agent of haplomycesis from the genus *Haplosporangium* and created a new genus, *Emmonsia*, with a single species *E. parva*. EMMONS and JELLISON (1960) reported the discovery of another species of this genus, *E. crescens*, characterized by the considerably larger size of the spherules. The authors proposed the term adiaspores for the spherules and, accordingly, adiaspiromycosis for the disease.

Numerous later studies showed that *E. crescens* is a pulmonary parasite of widespread distribution in countries of both moderate zones. It has also been recorded from Czechoslovakia by PROKOPIČ, DVOŘÁK, OTČENAŠEK 1965; OTČENAŠEK, DVOŘÁK, PROKOPIČ 1965. Infection may occur in numerous mammal species such as rodents, insectivores, edentates, marsupials, carnivores, rabbits and hares. The various modes of reproduction and circulation of *E. crescens* have been described in comprehensive ecological studies (DVOŘÁK, OTČENAŠEK, ROSICKÝ 1966; ROSICKÝ, DVOŘÁK, OTČENAŠEK 1967); the infection itself has been listed among the diseases with a natural focus of infection. The occurrence of *E. crescens* in man has been de-

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monstrated beyond doubt in 1964 (Doby-Duboiss et al.; Chevrel et al. 1964) when an occasional adiaspore was found in the lungs of a patient suffering from pulmonary aspergillosis. Of the second case of infection in man a brief account is given by Emmons in his introduction to Jellison’s monograph on adiaspiromycosis (1969). The third, and perhaps the first case of clinical disease due to heavy infection with E. crescents in man has been diagnosed in Czechoslovakia (Hálek et al. 1970). Masses of adiaspores have been recovered in the lung biopsy; tissue reaction was conspicuous and corresponded with the chest films and the clinical symptomatology.

Systematic studies of occasional parasitic and pseudoparasitic granulomas in the lungs of man revealed some particular spherical bodies of identical type which remained unidentified for a long time. However, when clinical adiaspiromycosis in man had been found in Czechoslovakia, these bodies were compared with adiaspores from the lungs of feral rodents and finally identified as adiaspores of the fungus Emmonsia crescents.

MATERIAL

The adiaspores in the lungs were found in 5 necropsies from Šikl’s Department of Pathology, Medical Faculty, Plzeň. Short abstracts of the autopsy reports are given.
Case no. 1. M. P., a 55 year-old housewife (autopsy no. 331/67, histol. no. I-6708, collection no. 716) died of myocardial infarct. In one of the blocks from the lungs a corpuscle in a fibrous encapsulation was found under the pleura; it resembled a “poppy seed” and stained with haematoxylin (Plate I, Fig. 1).
Case no. 2. J. P., a 62 year-old worker (autopsy no. 327/52, histol. no. 2811, collection no. 894) with carcinoma of the urinary bladder died of pulmonary emboli. One block with lung infarct contained a minute nodular granuloma. Further sections revealed an “ascaris” surrounded by purulent exudate and encapsulated by concentrically arranged fibrous connective tissue (Plate I, Fig. 2).
Case no. 3. M. H., a 62 year-old woman, former saleswoman (autopsy no. 831/68, histol. no. I-7781, collect. no. 790) with history of hypertensive disease died of pulmonary emboli. One block of the

Table 1. Comparison of measurements of the adiaspores and the layers of their wall in the lungs of man with adiaspores in the lungs of the field-mouse (in μ)

<table>
<thead>
<tr>
<th>Origin of adiaspore</th>
<th>Diameter of adiaspore</th>
<th>Layers</th>
<th>Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>superficial refractile</td>
<td>subsuperficial structural</td>
</tr>
<tr>
<td>Field-mouse</td>
<td>335</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Case no. 1</td>
<td>345</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Case no. 2</td>
<td>320</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Case no. 3</td>
<td>220</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Case no. 4</td>
<td>130</td>
<td>1</td>
<td>more than 1</td>
</tr>
<tr>
<td>Case no. 5</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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lungs showing dispersed catarrhal bronchopneumonia, revealed a spherical foreign body in the wall of a respiratory bronchiolo. The body was surrounded by giant cells and epitheloid cells (Plate I, Fig. 3).

Case no. 4. L. Ž., a 36 year-old woman (histol. no. 3101/42, collect. no. 893, from the Department of Pathology in České Budějovice) died of interstitial fibrosis of the lungs after delivery. The retrospective study revealed in one of the lungs blocks two neighbouring nodular granulomas of similar appearance. In the center of one nodule a cut section of a layered spherical but interrupted membrane was found, showing basophilic structures in its inside (Plate I, Fig. 4).

Case no. 5. J. P., a 52 year-old mining engineer (autopsy no. 859/59, histol. no. I-1225, collect. no. 895) with silicosis of the lungs (2nd stage) died of brain softening. In the lungs, old silicotuberculous lesions and a minute resorptive granuloma surrounding a foreign body were found (Plate I, Fig. 5).

Serial sections were cut from the blocks with suspicious bodies and stained with haematoxylin eosin and the Weigert van Gieson method; calcium was demonstrated with the von Kossa technique.

**FINDINGS**

The adiaspore of case no. 1 had a diameter of 345 μ and was situated in a hyaline nodule without any inflammatory infiltrate. The layered wall (overall thickness 42 μ) consisted of a superficial refractile layer and of the wall proper divided into a subsuperficial structural layer and a homogeneous layer; their thickness ratio was approximately 1 : 2 but was not uniform as illustrated in Plate II, Figs. 1 and 6. The thickness of the structural layer appeared to be more constant. The thickest part of the wall proper measured 27 μ. To the inner side a distinct basophilic layer was attached which, in its narrowest part measured 15 μ in breadth. In this layer von Kossa's technique gave a feeble, but undoubtedly positive result. The central part of the adiaspore was optically empty.

The adiaspore of case no. 2 measured 320 μ in diameter. Its wall (40 μ width) was of the same structure as that of the adiaspore of case no. 1 and also its individual layers were of similar proportions (Table 1, Plate II, Fig. 5). The basophilic inner layer showed evidence of protuberances on its inner surface; otherwise the centre of the adiaspore was empty. The adiaspore was surrounded by exudate from neutrophiles only, the outer layer of encapsulation consisted of maturing connective tissue.

The adiaspore of case no. 3 had a diameter of 220 μ. The wall was 27 μ thick and lacking of an innermost basophilic layer (Plate II, Fig. 4). The thickness of the individual layers is given in Table 1. When examining the granuloma enclosing the adiaspore in a series of sections, this seemed to have originated in the wall of a branching respiratory bronchiolo; the granuloma consisted of cells which appeared more like histiocytes than epitheloid cells in view of the missing palisading pattern. Multinucleate giant cells were attached to the surface of the adiaspore.

In case no. 4 the ruptured spherical body measured approximately 130 μ in diameter. The content was basophilic and more markedly condensed at the inner side of the wall. The wall was 6 μ thick, the proportions of its layers resembled these of the typical adiaspore (Table I, Plate II, Fig. 2). The adiaspore in the centre of the nodule was surrounded by neutrophiles, the periphery consisted of
differentiating histiocytes with some eosinophiles and plasma cells. Although additional histological sections were examined, the central portion of the other nodule could not be distinguished.

Also the superficial membrane of the body in case no. 5 was interrupted. The membrane was cut more or less tangentially, its width seemed to be approximately 4 μ, the diameter of the whole body 100 μ. Its content was eosinophilic, not homogeneous; at the site where the membrane was ruptured, cells of the tissue reaction were found directly on its surface. The adiaspore was enclosed in a granuloma consisting of an inner layer of epitheloid cells and maturing connective tissue at the periphery. The empty spaces between the epitheloid cells had been occupied earlier by cholesterol crystals.

The material of the present series was too limited and did not allow examination with a wider range of histological and histochemical methods. Standard methods enabled only an evaluation of the staining intensity of the individual layers. This was found to be highest in the superficial refractile membrane, less high in the structural layer and low in the homogeneous layer. The layers stained with eosin and picrofuchsin; an intensification of staining with haematoxylins was negligible in the layers of the proper wall, but was very prominent in the innermost layer in case no. 1 and 2.

DISCUSSION

For identifying the described fungus occasionally found in the lungs of man, its morphology has been compared with that of the typical adiaspores from an infected field-mouse*). Adiaspores of about the same size have been selected on purpose (Plate II, Fig. 3). Table I and Plate II demonstrate the striking resemblance of the fungal bodies from case no. 1—3 and the adiaspore from the field-mouse. There was only one difference: the adiaspore from the field-mouse had a clearly visible granular layer on the inner side of the wall and the cavity was occupied by cytoplasmatic structure, while the adiaspores in our human cases were hollow and apparently not viable, by contrast to the adiaspores of the field-mouse. The comparison revealed that the distinct inner basophilic layer found in

*) We wish to express our thanks to Doc. Dr. K. Kučera for supplying us with the first comparative material.
the adiaspores of cases no. 1 and 2 is not a regular component of the wall and seems to be the collapsed necrotic and calcified remnants of the cytoplasmatic content of the adiaspore. Our conclusions are in accord with the various figures in Jellison’s monograph (1969) and are illustrated schematically in Fig. 1. Also the fungal bodies in the cases no. 4 and 5 seem to be growing adiaspores.

The different histopathological reaction in the individual cases is remarkable. Although it is impossible to draw any definitive conclusions on the pathogenicity of the adiaspores and on the course of tissue reaction solely on the grounds of our findings, it may be stressed that the latter is not a simple granulomatous process encapsulating a foreign body. The evidence of purulent exudation is in agreement with the data cited by Jellison (1969) from the work of Ashburn and Emmons.* These authors concluded from the results of experimental infection with E. parva that the lesions caused by the adiaspores of this species are more than a foreign body reaction. In some of the granulomas as well as in older lesions they observed a prominent infiltration of neutrophilic leucocytes, particularly immediately adjacent to the cells of the fungus. The histopathological reaction in case of Doby-Dubois et al. (1964) was different from our findings. It is possible to compare this with our case No. 3, but the process was more progressive.

At histopathological examination, the adiaspores have been considered to be sections of worms (this also happened in our case no. 2) or their eggs. They have also been diagnosed as aspirated exogenous foreign bodies, especially when the structure of the adiaspores had been damaged. Increased attention will certainly lead to more findings of adiaspores in man and, hence, to the gradual disclosure of its complicated mode of infection.

REFERENCES


*) Arch. Pathol. 39: 3—8, 1945.


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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Subpleural hyaline nodule with an adiaspore in case no. 1. Fig. 2. Section through an adiaspore surrounded by neutrophilic exudate inside the maturing connective tissue in case no. 2. Haemorrhagic content of the adjacent lung alveoli. Fig. 3. Granuloma with an adiaspore (case no. 3) protruding into the lumen of the bronchiole; serous exudate with spaces of dissolved cholesterol crystals in the bronchiolar lumen. Fig. 4. Two nodular granulomas in case no. 4. The center of the upper nodule is occupied by neutrophilic exudate harbouring a young adiaspore. Fig. 5. In the minute nodule (case no. 5), epitheloid cells enclose a minute adiaspore with a disturbed superficial membrane (top), the slit (bottom) is due to the retraction of the contents from the membrane. Figs. 1, 2, 5 — haematoxylin-cosin; Figs. 3, 4 — Weigert-van Gieson (×60).

Plate II

Fig. 1. Adiaspore in case no. 1 (×125). The thickest part of the homogeneous layer is marked by an arrow; the thinnest part of the layer is situated at the site the close-up picture of which is figured in Fig. 6. Haematoxylin-cosin. Fig. 2. A ruptured adiaspore in case no. 4 surrounded by neutrophiles. Weigert-van Gieson. Fig. 3. The wall of the adiaspore in the lung of a field-mouse. Trichrome. Fig. 4. The wall of the adiaspore in case no. 3; Fig. 5. — case no. 2; Fig. 6. — case no. 1; Figs. 2—6 (×500). 1 — superficial refractile layer, 2 — subsuperficial structural layer, 3 — homogeneous layer, 4 — granular layer on the inner side of the wall, 5 — plasmatic content of the adiaspore, 6 — basophilic layer on the inner side of the wall.