found that 15 of 23 IDD lymphocyte preparations showed at least 21 percent insulinoma cell death as compared to none in 12 normal controls. Among positive cases, 2 showed predominantly lymphocyte-mediated cytolysis, 2 showed antibody-dependent lymphocyte cytolysis, and 11 demonstrated that both cytotoxic mechanisms were operating. In our test system, serum plus complement had no effect on cytolysis (Fig. 3).

In order to study the cytotoxic activity of subpopulations of lymphocytes, cells from seven patients with positive reactions were further fractionated with sheep erythrocytes and Ficoll-Hypaque gradient into rosette-forming cells and non-rosette-forming cells. Those lymphocytes which have receptors to form sheep erythrocyte rosettes in 2 hours at 4°C were operationally defined as T lymphocytes, while non-rosette-forming cells were defined as bone marrow–derived lymphocytes (B cells) or cells of third population with Fc receptors (K cells) (*12*). The assays were performed with insulinoma cells cultured with rosette-forming cells alone or non-rosette-forming cells plus serums. The two patients who showed predominant lymphocyte-independent cytolysis had a significant enhancement of cytotoxicity by T cell enrichment. Another patient, who showed antibody-dependent lymphocytolysis, had enhanced cytolysis with non-rosette-forming cells plus serums. The other four cases showed cytotoxicity of equal degree by two subpopulations, indicating both antibody-independent (T cells) and antibody-dependent (B or K cells) lymphocyte-cytotoxic processes.

Our study indicates that lymphocytes, and not antibodies, in IDD patients are the primary aggressors in the process of pancreatic β cell autoaggression. Thus, cell-mediated immunity against β cells may be an important pathogenic mechanism in IDD. These findings would explain the pancreatic lesions of IDD that are characterized by marked infiltration of mononuclear cells, which are commonly seen in other autoimmune endocrine diseases. The fact that one patient already showed T cell cytotoxicity even before insulin therapy may imply that the sensitization to insulin is not a primary factor. One intriguing question which remains to be answered is whether infectious agents, particularly viruses (*13*), might trigger this process of autoimmune β cell destruction in a genetically predisposed individual. This possibility needs further exploration since, in the future, preventive measures might be developed for IDD.

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18 November 1975

Palaeosclerotium, a Pennsylvanian Age Fungus Combining Features of Modern Ascomycetes and Basidiomycetes

Abstract. *Fruiting bodies described previously as sclerotia of* Palaeosclerotium pusillum *are cleistothecium-like, enclosed structures, containing spores within an ascus. The cleistothecia are composed of hyphae having dolipore-like septa and are attached to vegetative hyphae having clamp connections. The unique combination of ascomycete-like reproductive bodies and basidiomycete-like hyphae present in this Pennsylvanian fungus suggests that there already existed, during the Middle Pennsylvanian period, a group of fungi intermediate between the Ascomycetes and Basidiomycetes.*

Permineralized fossil fruiting bodies found associated with Pennsylvanian plants (*1*) combine features of the traditional classes of higher fungi, Ascomycetes and Basidiomycetes. The cleistothecial fruiting body (Fig. 1A) and saclike ascus (Fig. 1A, arrow; enlarged in Fig. 1B) are characteristic features of sexual reproduction in the class Ascomycetes. In contrast, the clamp connections (Fig. 1C) and complex septal pores (Fig. 1F) identify the hyphae with the class Basidiomycetes.

The fruiting body illustrated is a small, spherical, enclosed (cleistothecium-like) structure (624 μm in diameter) composed of a compact outer peridium (112 μm thick) of pseudoparenchymatous tissue and a central zone (400 μm in diameter) of polyhedral tissue (Fig. 1A). The peridium of pseudoparenchymatous cells is continuous to the exterior with vegetative hyphae (Fig. 1, A and C). The central polyhedral tissue (Fig. 1A) is composed of ramified hyphae that average 3.5 μm in diameter (Fig. 1E). Therefore, the fossil form has a dense, compact, two-zoned spherical fruiting body situated within a mycelium. The fossil resembles structures found within the ascomycete family Trichocomataceae (*2*).

The vegetative mycelium is most remarkable because of the presence of clamp connections. A number of such clamp connections are present at the cross walls of the hyphae, which organically attach to the pseudoparenchymatous cells of the outer zone of the fruiting body (Fig. 1C). One well-preserved clamp connection is seen to have features commonly recognized in clamp connections of extant basidiomycete fungi (Fig. 1C, arrow). Two septa are present; one is positioned within the base of the clamp and the second partitions the hypha just below the opening circumscribed by the clamp.

The central, polyhedral tissue of the fruiting body contains numerous sporangia (Fig. 1, A, B, and D). These globose sporangia are small, ascus-like structures measuring from about 31 μm (Fig. 1B) to about 39 μm in diameter (Fig. 1D). The spores are initially produced and remain within the sporangium, similar to the way in which ascospores are produced within asci. Developmental stages in spore production are preserved within this specimen. While some of the sporangia contain mature spores (Fig. 1B), others demonstrate nonsynchronous spore development with marked differences in size and appearance of spores (Fig. 1D, where three mature spores are seen in section at the lower right and immature spores are seen at the left). With increasing maturity, the sporangial wall breaks and the spores are released into the spaces between the hyphae that make up the polyhedral tissue (Fig. 1B, arrow). The number of spores produced within a sporangium is inconsistent; however, four and eight are most commonly encountered when an entire sporangium is studied in serial sections (Fig. 1B is an example with four spores and Fig. 1D an example with eight spores). The mature spores average 11.9 μm in diameter. Sections seen with light optics suggest that the spores are ornamented with short spines (Fig. 1B). However, sections of walls seen with the scanning electron microscope (SEM) demonstrate that this apparent surface ornamentation results, at least in part, from an optical effect produced by the passage of light through the complex internal layerings of the spore wall (Fig. 1D, central mature spore).

In contrast to the septa with associated clamp connections present on the vegetative hyphae, clampless septa occur within the hyphae forming the central polyhedral tissue of the fruiting body (Fig. 1E, arrow and enlarged inset). Located on the peripheries of the pores traversing these septa are wall thickenings, which are interpreted as the fossilized remains of dolipores (Fig. 1E, inset, shows lateral view; Fig. 1F shows face view). In extant fungi, dolipores are relatively complex pores characteristic of the septa of dikaryotic hyphae of some basidiomycetes (*3–5*), in contrast to the simple pores of ascomycete septa.

The identification of clamp connections and the demonstration of the organic connection of hyphae bearing these clamp connections to a body composed of hyphal elements support the interpretation that such bodies are fruiting structures. The clamp connection is a structure, found only on a dikaryotic hypha, which allows paired mating nuclei (the dikaryon) to replicate and be partitioned as new pairs into each new segment of hypha (*4*). The dikaryon is needed at the site of fruiting body production in order for sexual reproduction (karyogamy and meiosis) to occur. Therefore, a dikaryotic mycelium has the potential for sexual reproduction, and any problematic fossil body constructed of dikaryotic hyphae might be a fruiting body. An alternative suggestion for this fossil is that the various structures described here might represent more than one organism. The fossil fruiting body (Fig. 1A) could be interpreted as an ascomycete ascocarp parasitized by a neighboring mycelium of a basidiomycete. We know that the Carboniferous flora contained fungi that have mycelia bearing clamp connections (*6*). However, both the organic continuations of hyphae bearing clamp connections to the pseudoparenchymatous tissue (Fig. 1C) and the presence of complex septa within the central polyhedral tissue (Fig. 1, E and F) demonstrate that such an interpretation is unlikely and that a single

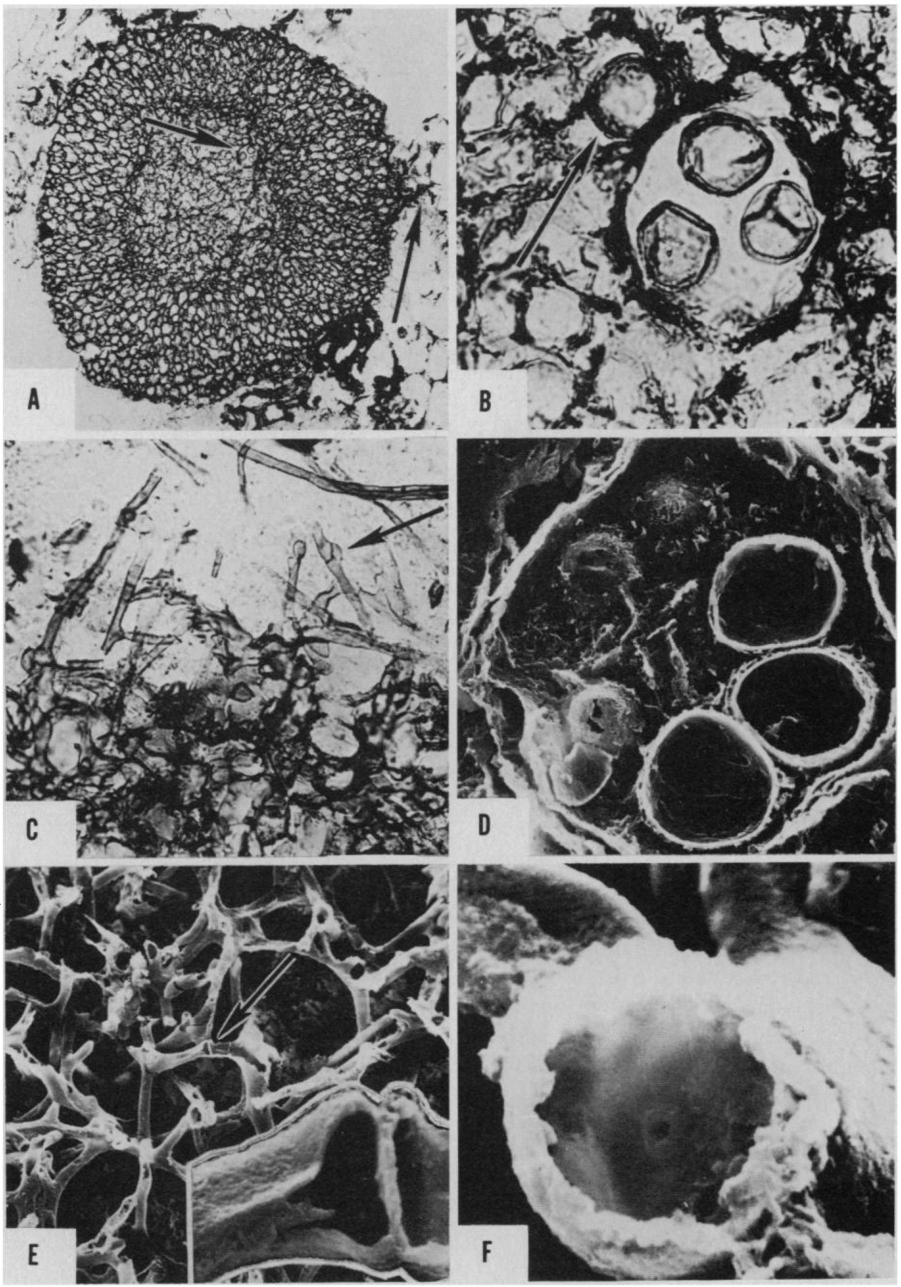

Fig. 1. *Palaeosclerotium pusillum*. (A) Fruiting body with ascus (interior arrow) and mycelium (exterior arrow) (light optics, × 90). (B) Meiosporangium and meiospores (light optics, × 900). (C) Pseudoparenchymatous peridium and attached hyphae with clamp connections (light optics, × 510). (D) Ascus with three sectioned mature spores and also young spores (SEM, × 1500). (E) Polyhedral tissue, showing hypha with septum (arrow and enlarged inset) (SEM, × 900 and × 9000). (F) Polyhedral tissue; septum and pore in face view (SEM, × 11400).

organism is probably involved. In addition, the presence of dikaryotic hyphae (Fig. 1C) and internally born sporangia containing four and eight spores (Fig. 1, B and D) suggests that the body (Fig. 1A) is a sexual reproductive structure and not a sclerotium as previously reported (*7*).

Sterile mycelial remains, with septa, of higher fungi are occasionally encountered in excellent states of preservation in Carboniferous strata (*8*); however, only on rare occasions do specimens exhibit reproductive stages of the types that are diagnostic of various extant groups (*6*). The discovery of such reproductive structures that characterize the advanced fungi in the Paleozoic provides a time scale on which various concepts of phylogeny of the Ascomycetes and Basidiomycetes may be judged. *Palaeosclerotium pusillum* may represent a fungus having basidiomycete affinities but retaining reproductive structures similar to those of ascomycete progenitors rather than having a basidium and basidiocarp. Dikaryotic vegetative hyphae, clamp connections, and complex septal pores occur ubiquitously throughout taxa of extant Basidiomycetes; therefore, the immediate ancestors of the Basidiomycetes might be expected to have these features. Alternatively, *Palaeosclerotium* might represent an early occurrence of an ascomycete having clamp connections and complex septa. Some workers have suggested that the ascomycete crosier is homologous to the clamp connections of basidiomycetes, and some species of *Tuber* are reported to have a dikaryophase and true clamp connections (*9*). In addition, some ascomycetes apparently produce complex septa in their ascogenous hyphae (*5*). The combination of ascomycete reproductive features and complex vegetative features characteristic of basidiomycetes suggests that a group of fungi intermediate to Ascomycetes and Basidiomycetes existed early enough in the fossil record to have been ancestral to extant fungi.

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18 December 1975; revised 26 January 1976

Acid Precipitation and Embryonic Mortality of Spotted Salamanders, *Ambystoma maculatum*

Abstract. *Spotted salamanders breed in temporary pools formed in early spring by melted snow and rain. Many of these pools reflect the low* pH *of precipitation in the northeastern United States. Egg mortality is low (< 1 percent) in pools near neutrality, but high (> 60 percent) in pools more acid than* pH *6. Developmental anomalies and the embryonic stage at which death occurs are the same in field situations as at corresponding* pH*'s in laboratory experiments.*

The deleterious effects of acid precipitation on forests and fisheries have been a source of concern for two decades in Scandinavia and more recently in North America (*1*). Acid precipitation has lowered the *p*H of lakes and streams, but temporary ponds that form by accumulation of melted snow or rain may be still more vulnerable to alteration of *p*H because precipitation entering them has little contact with buffer systems and is not diluted by mixing with standing water (*2*). Temporary ponds are important breeding sites for a number of species of frogs, toads, and salamanders, and these amphibians may be the vertebrate animals most immediately and directly affected by acid precipitation.

Laboratory studies (*2*) showed that embryos of Jefferson and spotted salamanders (*Ambystoma jeffersonianum* and *A. maculatum*) are sensitive to *p*H. Hatching success of 90 percent or more was achieved by Jefferson salamanders only at *p*H 5 and 6, and by spotted salamanders only at *p*H 7, 8, and 9. Beyond those limits, mortality rose sharply and was associated with distinctive embryonic malformations. Many of the natural breeding sites of spotted salamanders in the Ithaca, New York, region are acidic. The *p*H of 17 temporary pools was measured during April and May 1975, the period of egg deposition and development (*3*). Fourteen pools had a *p*H of 6 or less, only one had a *p*H of 7. In contrast, only one of 13 permanent ponds in the same area had a *p*H below 6, and ten were *p*H 7 or higher (*4*).

Laboratory data indicate that acidity measured in some breeding ponds should cause embryonic mortality at specific stages of embryonic development in spotted salamander eggs. This prediction was tested under field conditions by following development of spotted salamander eggs in five ponds on Connecticut Hill, Tompkins County, New York. The ponds are within a circle 1 km in diameter and span an altitudinal range from 460 to 625 m. At the time eggs were laid, pond *p*H's ranged from 4.5 to 7.0; by hatching, the *p*H had increased by 0.25 to 0.5 *p*H units in each pond. Water temperatures rose from 8° to 23°C during development (*5*). Temperature did not differ significantly among the ponds ($F_{4,33} = 0.07$, $P > .05$).

Every egg that could be found in each pond was examined at intervals of 3 to 7 days. The stage of development was determined and the number of live and dead eggs in each clutch was recorded (*6, 7*). Most clutches were counted several times during the study, but no clutch was counted twice at the same embryonic stage. Dead eggs decay and disappear over a period of days; thus, this index is an estimate of the minimum cumulative mortality that had occurred by a given stage of development.

An abrupt transition from low to high embryonic mortality occurred below *p*H 6 (Fig. 1). Mortality reached a maximum of 0.66 percent at *p*H 7 (pond A) and 0.91 percent at *p*H 6 (pond B). At *p*H 5.5 (pond C) maximum mortality rose to 43.7 percent and at *p*H 5.0 and 4.5 (ponds D and E) exceeded 65 percent. Mortality was low in all ponds during early embryonic development. Even at *p*H 4.5 (pond E) mortality did not exceed 1.3 percent through late gastrulation. In all ponds mortality increased at neurulation and again at late stages of gill development and at hatching. These observations are strikingly similar to laboratory observations (*2*), both in the levels of mortality at different *p*H's and in the embryonic stages at which death occurred. This similarity strongly suggests that the acidity