indicate that only a small fraction of the $^{65}Zn^{2+}$ taken up by cultured rat islets is associated with the storage granules (*21*). These findings, in addition to the results presented in this report, point to a more important role for Ca^{2+} in the storage of insulin than was previously supposed.

Finally, we note that the Zn^{2+} binding site (site I in Fig. 1) is not present in insulin from certain species (for instance, guinea pig and coypu); however, the carboxylate groups (either Glu or Asp) (*1*) at B-13 which form site II are conserved in all insulin species sequenced to date (*2*).

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1. Abbreviations: Glu, glutamic acid; His, histidine; Asp, aspartic acid; B-13 and B-10, B-chain positions 13 and 10, respectively.
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23. Bovine insulin was purchased from Sigma, and divalent metal ions were removed by Chelex-100 ion-exchange resin as previously described [M. F. Dunn, S. E. Pattison, M. C. Storm, E. Quiel, *Biochemistry* **19**, 718 (1980)]. Concentrated metal ion–free insulin solutions were prepared by dissolving lyophilized insulin in twice-distilled water. The *p*H was raised to 10.5 with NaOH to effect complete solution and was then carefully lowered to 8.00 with dilute $HClO_4$. Cadmium-113 oxide (isotopic purity, 96 percent; Oak Ridge National Laboratory) was dissolved in H_2SO_4 and neutralized with NaOH to *p*H 6.0. Stock solutions of $CoCl_2$ and $Zn(NO_3)_2$ were purchased from Alfa Products as the atomic absorption standards. The NMR spectra were obtained at ~ 19.97 MHz, using a modified multinuclear Bruker WH90D-18 (18-inch, 2.11-T magnet) with quadrature phase detection for all nuclei. The probe has a homemade insert designed for sample tubes of 15-mm outer diameter (containing 5 ml of solution) and an external D_2O field/frequency lock. The sweep width was 15 kHz, acquisition time was 0.27 second (4K real data points), and exponential multiplication producing line broadening of 10 Hz was employed. The 90° pulse was 12.5 μsec for a pulse power of 25 watts, and a flip angle of 30° was employed. No proton decoupling was used.

24. Supported by NSF grant CHE-7811548, NSF grant BNS76-20218-01, PHS grant 1R01 GM25877, American Diabetes Association grant 40660, and a grant from the UCR Committee on Research. The UCR Bruker WH90D-18 multinuclear Fourier transform NMR spectrometer was provided by NIH Biomedical Sciences grant 5S05RR 07010-09 and NSF grant MPS75-06138. We are grateful to G. G. Dodson for helpful discussions and for sharing his unpublished data with us.

18 November 1980

Evidence for Endomycorrhizae in Pennsylvanian Age Plant Fossils

Abstract. *Fossil fungal chlamydospores from the tissues of a number of Pennsylvanian age fossil plants are described. Features such as size, shape, wall organization, and the structure of the subtending hyphal stalk suggest affinities with the modern endomycorrhizal fungus* Glomus.

Paleobiological studies suggest that the fungi are an ancient group that may have been among the earliest life forms to have evolved. Filamentous fragments that may represent hyphal remains and possible fungal spores were identified from Precambrian sediments (*1*). Despite the uncertainties in accurately identifying these Precambrian remains, unquestionable fungi representing all of the major divisions are known from the Paleozoic to the Recent (*2*).

Of the Paleozoic fungi that have been described (*3*), several different forms were identified in the petrified plant material that accumulated during the formation of the Carboniferous coal swamps. Although fungi are known to have oc-

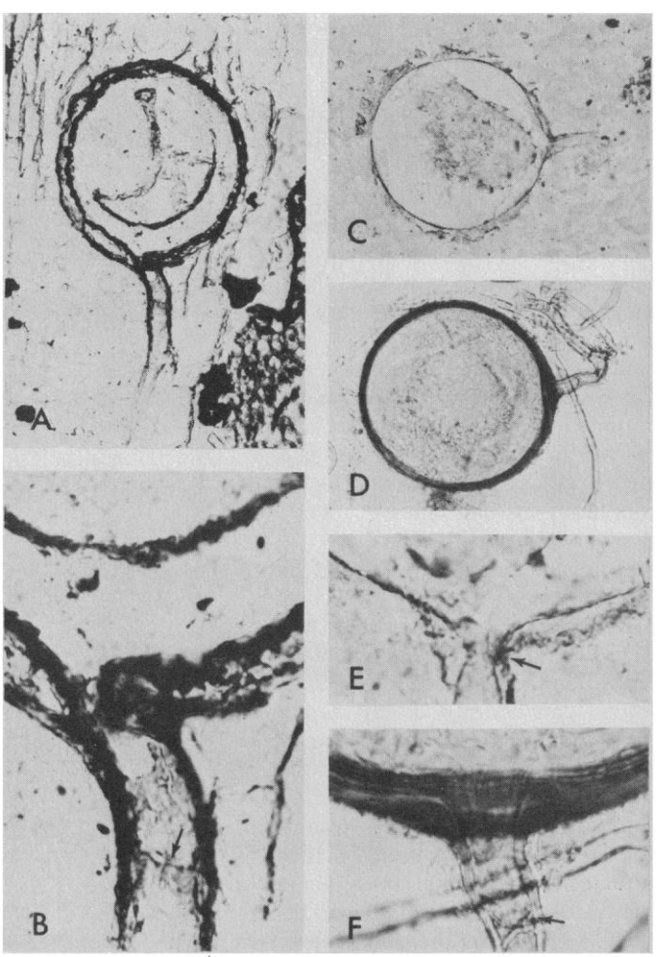

Fig. 1. Endomycorrhizal chlamydospores. (A) Fossil chlamydospores showing the stalk and multilayered wall (× 160). (B) Hyphal attachment to fossil chlamydospore showing curved occlusion in the stalk (arrow) (× 740). (C) Fossil chlamydospore with simple hyphal attachment (× 160). (D) *Glomus mosseae* chlamydospore (× 160). (E) Simple hyphal attachment of fossil chlamydospore showing thickening of wall (arrow) at spore base (× 740). (F) *Glomus mosseae* chlamydospore with curved septum in the stalk (× 740).

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curred with the plants that make up these sediments, few studies have been undertaken with these organisms because of the lack of definitive character suites upon which to make comparisons with living forms.

We describe chlamydospores of Pennsylvanian age (*4*) that are morphologically and structurally identical with those produced by living endogonaceous (*5*) fungi assigned to the genus *Glomus*. The fossil chlamydospores occur singly (Fig. 1, A and C) and in loose clusters throughout the plant matrix, but they are most frequently encountered in the cortical tissues of the roots and underground organs of arborescent lycopods, cordaites, and the marattialean tree fern *Psaronius*. The majority of the spores are spherical and range from 100 to 400 μm in diameter. The wall is about 10 μm thick and constructed of two to three distinct wall layers (Fig. 1B). The outer surface is smooth.

Several fossilized spores contain a portion of the nongametangial hypha upon which they were formed (Fig. 1A). Hyphae range from 10 to 20 μm wide, increasing in diameter to 30 μm near the spore to form a funnel-shaped stalk. The outer wall of the hyphal stalk is continuous with the wall of the spore, and the contents of the chlamydospore are separated from the hypha by a curved inclusion in the stalk (Fig. 1B). A number of spores were also found to possess a simple, unoccluded stalk with a constricted opening at the spore base (Fig. 1E).

On the basis of the morphology and structure of the spores (*6*) and the funnel-shaped hyphal stalk with curved inclusion (Fig. 1, D and F), we believe that these fossil remains have affinities with the fungus *Glomus*. *Glomus* chlamydospores are borne terminally on single (Fig. 1D) undifferentiated, nongametangial hypha either in sporocarps, loose in open clusters, or singly in the soil (*7*). At maturity the chlamydospores may be separated from the subtending hypha by a septum which may consist of a thin membrane at the base of the spore, or a curved extension of the inner wall of the stalk (Fig. 1F). Studies of the living *Glomus* chlamydospores from different geographic regions (*8*) indicate that a number of features (spore size, shape, and stalk structure) are variable within a species, and this same variability appears to exist among the fossil specimens collected at different localities.

Interest in the genus has centered on its role in the production of vesicular-arbuscular endomycorrhizae. These fungi may be found in most habitats in nature associated with an extremely diverse group of plants, including those that inhabit swamps and marshes and even some aquatics that have been regarded as being nonmycorrhizal.

The material described here is the most convincing evidence assembled to date that establishes the presence of modern endomycorrhizal fungi as early as the Pennsylvanian. The existence of well-preserved fungal chlamydospores in the underground organs of a number of Carboniferous vascular plants provides an opportunity to investigate both the biology and evolutionary history of a mycorrhizal fungus that is exceptionally cosmopolitan today. The identification of endomycorrhizal fungi in Carboniferous plants also affords an opportunity to consider questions of a more biological nature, such as some of the intermediate stages in the evolution of the mycorrhizal system.

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5. Modern members of the Endogonaceae currently include seven genera characterized by the organization of the reproductive structures. Living species of *Glomus* possess vesicular-arbuscular mycorrhizae surrounded by a hyphal network that extends into the soil and penetrates the host plant. Both short-lived arbuscules and vesicles may be produced and, under adverse conditions, such as the death of the host, endotropic chlamydospores are the only structures able to survive.
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17 October 1980

Alternative Male Strategies: Genetic Differences in Crickets

Abstract. *Male field crickets,* Gryllus integer, *call and attract mates, or they silently intercept females attracted to calling males. Selection experiments demonstrate that the duration of nightly calling has an important genetic component. Mean calling times in high and low lines were significantly different and had realized heritabilities of 0.50 and 0.53, respectively. Selection can operate in such a way that each of the alternative forms of male reproductive behavior is associated with a specific genetic substrate. This has not yet been shown for other species in which males adopt contrasting modes of mating behavior.*

Conspecific males in the same population may have different types of mating behavior. Some male field crickets, *Gryllus integer*, call very regularly and attract females, whereas other males (satellites) call infrequently, or not at all, and intercept females attracted by the calling of neighboring males (*1*). The male *G. integer* show definite tendencies for calling or for satellite behavior (*2, 3*), but population density, male aggression, and time since sunset influence the duration of calling (*2–4*). Similar patterns occur in vertebrates and other invertebrates (*2, 3, 5, 6*), and a genetic model has been proposed to explain the coexistence of alternative male reproductive strategies (*7*). There is, however, no direct evidence that this type of variation in male behavior has a genetic component. I now report the results of selection experiments demonstrating that male field crickets differ genetically with respect to the amount of time they call each night.

Gryllus integer were raised in the laboratory from eggs laid by females that were collected in San Antonio, Texas, during July 1979. After the final molt, males were placed in separate 4.2-liter glass jars fitted with sound-operated relays to monitor the total calling time each night (*8*). In the initial generation, males were monitored for 21 to 45 days. Most males began calling at 3 to 6 days of adult age, a time corresponding to the initial production of spermatophores (*9, 10*). There were nightly variations in individual calling times, but average calling did not correlate with age or weight of the males (*9*). Calling time was therefore computed as the average calling per night from 7 to 16 days of adult age for each male (*11*) (Fig. 1).

Two to four males from each end of

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