Table 1. Mean values for total and ester sulfate in three peat-forming areas.

| Sampling site | Percentage of sulfur (dry weight basis) | | Ester sulfate as a percentage of total sulfur |
|---|---|---|---|
| | Total | Ester sulfate | |
| Minnie's Lake (freshwater)* | 0.19 | 0.047 | 24.73 |
| Chesser Prairie (freshwater)* | 0.18 | 0.045 | 25.00 |
| Little Shark (marine)† | 5.16 | 1.22 | 23.64 |

*Mean of 5 samples. †Mean of 14 samples.

ences in the total sulfur content between the two freshwater peats is quite small; (iii) ester sulfate represents approximately 25 percent of the total sulfur; (iv) there is little difference between the freshwater and marine peats in the ester sulfate content as a percentage of the total sulfur; (v) the ester sulfate content increases with the depth of sample; and (vi) the ester sulfate content closely parallels the carbon-bonded sulfur and the total sulfur content as a function of depth.

It is difficult to hypothesize which naturally occurring compounds would be likely to have ester sulfate linkages. Ester sulfate linkages in substances such as choline sulfate, fucoidan, and chondroitin sulfate have been reported in various organisms and may eventually contribute ester sulfate to the sediment upon the death of the organism. Organic matter is not readily degraded in a water-logged swamp-marsh environment, and thus lignin-derived heteropolycondensates such as humic and fulvic acids can accumulate. These substances are known for their relatively high phenolic content (*9*); some of the phenolic groups may react with various sulfur forms to produce ester sulfate linkages. Sugars have been extracted from humic and fulvic acids, and these moieties may contain ester sulfate linkages (*10*). Thus ester sulfate may enter the sediment directly from the remains of certain organisms or it may be formed in situ if certain reactive chemical groups and sulfate are present.

Since peat is the first member of the coalification sequence, the relatively high values of ester sulfate in peat are significant. Ester sulfate incorporated in the peat-forming stage can be carried through the other stages of coal formation, or it can be added during subsequent stages. As far as we know, the ester sulfate content of lignites and bituminous and anthracite coals has not been investigated. Ester sulfate may be viewed as the form in which sulfate is stabilized geochemically. When a sediment is buried and becomes increasingly anaerobic, sulfate becomes an important source of oxygen for microbial metabolism. Hydrogen sulfide from the reduction of sulfate can then react with organic matter to form organic sulfur linkages. In a laboratory experiment gaseous hydrogen sulfide was shown to react with fatty aldehydes to form a variety of organic sulfur compounds (*11*). Thus, it is apparent that ester sulfate can be reworked in the sediment in such a way that it contributes to the total sulfur content in coal.

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## Chitinozoans from the Late Precambrian Chuar Group of the Grand Canyon, Arizona

Abstract. *Carbonaceous shales of the late Precambrian Chuar Group of the Grand Canyon, Arizona, contain abundant and well-preserved chitinozoans. The occurrence of these distinctive, tear- and flask-shaped microfossils, the oldest chitinozoans now known and the first to be reported from the Precambrian, seems to suggest that heterotrophic protists (or primitive metaozoans) were extant at least as early as about 750 ± 100 million years ago.*

One of the classic unsolved problems in paleobiology is the question of the origin of the Metazoa. In recent years, studies of megascopic fossils from strata of latest Precambrian and earliest Cambrian age have provided much new information regarding early stages of metazoan diversification (*1*). These studies suggest that megascopic invertebrates [and apparently also megascopic algae (*2*, *3*)] may have first appeared about 650 million years ago. Despite this recent progress, however, and although algal microbiotas of the earlier Precambrian

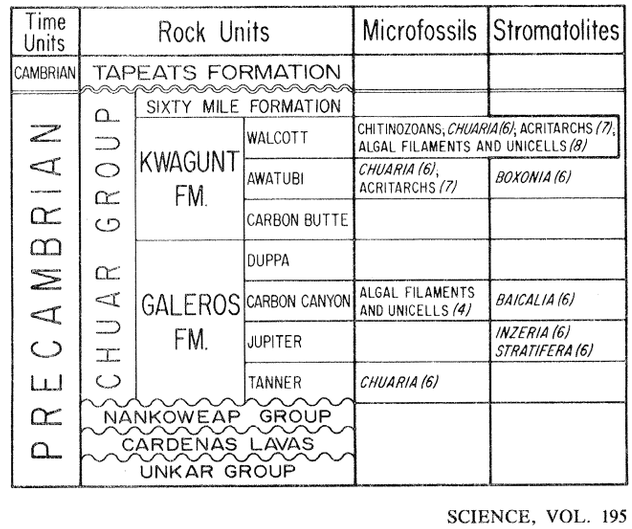


Fig. 1. Stratigraphic relationships of fossiliferous late Precambrian sediments in the Grand Canyon region of Arizona.

have similarly become better known in recent years (*4*, *5*), paleobiologic studies have as yet yielded little evidence of the heterotrophic protists from which metazoans were presumably derived. Indeed, among protozoans, the likely precursors of the Metazoa, rhizopods (namely, Foraminiferida) are first known only from the late Cambrian (*1*, *3*) and ciliates (namely, Tintinnida) and actinopods (namely, Radiolaria) date only from the Ordovician (*1*, *3*). In terms of cellular organization, there thus exists a gap in the known fossil record between the microscopic, unicellular autotrophs of the earlier Precambrian and the megascopic, multicellular heterotrophs of the latest Precambrian. We report here the discovery of Precambrian chitinozoans, planktonic animal microfossils, that in grade of organization may begin to bridge this gap in the known record of early evolution.

The chitinozoan-bearing samples studied here were obtained from the late Precambrian Chuar Group, exposed on the flanks of Nankoweap Butte, in the eastern Grand Canyon of the Colorado River, Arizona. As shown in Fig. 1, the Chuar Group includes three formations, two of which have been subdivided into a total of seven members (*6*); several types of stromatolites and plant microfossils have been reported from the sequence (*4*, *6*–*8*) (Fig. 1). The chitinozoans occur in carbonaceous shales of the Kwagunt Formation, 15 to 17 m above the "flaky dolomite bed" which marks the base of the Walcott Member (Fig. 1) (*6*). Chitinozoans have also been detected, but are of rare occurrence, in the immediately overlying cherty pisolite bed from which diverse microfossils have been reported (*8*). The chitinozoans are demonstrably indigenous to the Kwagunt sediments: they have been detected in samples collected at four localities, spaced over a distance of 0.5 to 1 km (*9*); they occur in both shales and cherts of the sequence (*9*); they have been detected both in acid-resistant residues (Fig. 2, A through G) and in petrographic thin sections of the Kwagunt rocks (Fig. 2, H through J); and they are of common occurrence in thin sections of the shales (having a calculated abundance of about 10,000 microfossils per cubic centimeter of rock).

The radiometric age of the Chuar Group is not well defined. The group underlies the "Great Unconformity" that marks the Precambrian-Cambrian boundary in the area; it overlies the Nankoweap Group, which, in turn, rests on the Cardenas Lavas, volcanic rocks which have a minimum radiogenic age of about

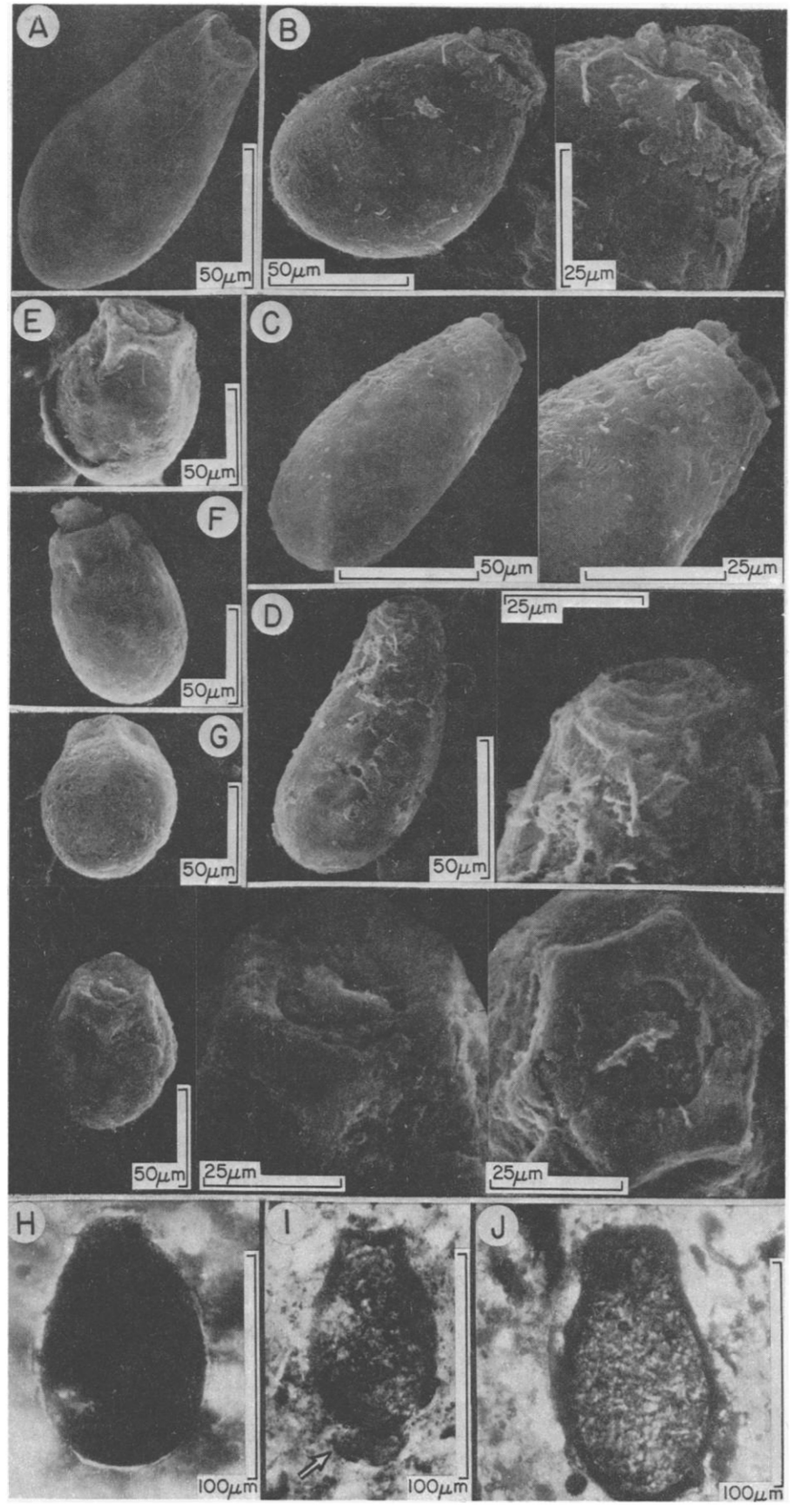


Fig. 2. Optical photomicrographs (H through J) and scanning electron micrographs (A through G) showing tear-shaped (A through D, H) and flask-shaped (E through G, I, J) chitinozoans in petrographic thin sections (H through J) and in acid-resistant residues (A through G) of carbonaceous shale from the late Precambrian Chuar Group (Kwagunt Formation, Walcott Member) of the eastern Grand Canyon, Arizona. The occurrence of an operculum-like body attached to the aboral pole of the flask-shaped vesicle shown in (I) (arrow) suggests that chitinozoans of this type may have formed chain-like colonies.

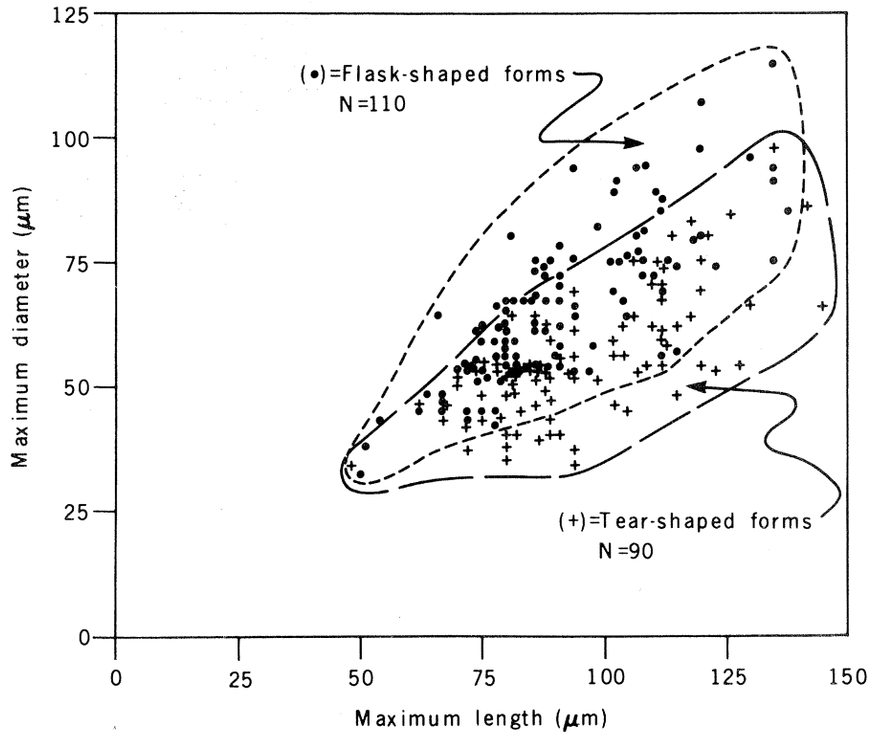


Fig. 3. Scatter diagram showing the size range of 200 chitinozoans measured in petrographic thin sections of Kwagunt shale.

850 million years and a probable age of about 1150 million years (Fig. 1) (*6*). Thus, the chitinozoan-bearing shales and cherts of the Kwagunt Formation are of late Precambrian age; being among the younger units of the Chuar sequence (Fig. 1), they seem likely to have an age of between about 650 and perhaps 850 million years.

In addition to organic-walled acritarchs (*7, 10*), two types of chitinozoans, flask-shaped (Fig. 2, E through G, I, J) and tear-shaped (Fig. 2, A through D, H), have been detected in the Kwagunt shales. The two varieties occur in roughly equal abundance; members of both forms are organic in composition, have psilate to granulate surface textures, and are structurally well preserved. The flask-shaped forms range in length from about 50 to 138 μm, with an average length of 91 μm (standard deviation = 19 μm; $N$ = 110) (Fig. 3); they have a length-to-width ratio of about 1.4 : 1. The vesicles are ellipsoidal to ovoidal in form and exhibit a short neck with a pronounced collar. The majority, and perhaps all, of these microfossils occurred singly rather than in chain-like colonies; although the occurrence of an operculum-like body attached to the aboral pole of one specimen (see arrow in Fig. 2I) suggests that chain formation may have occurred [see (*11*), plate 2, figure 15], studies of numerous specimens by scanning electron microscopy have failed to reveal the presence of basal scars that would provide firm evidence of coloniality. These microfossils are comparable in size, shape, surface texture, and general organization to the Ordovician and Silurian chitinozoan *Desmochitina* (for example, *D. minor* and *D. nodosa*) (*12*), although they appear to differ from such younger taxa in their characteristic hexagonal apertures and triangular opercula (Fig. 2, E through G). If found in the mid-Paleozoic, they would probably be regarded as representing a newly detected species of this genus (*13*).

The tear-shaped members of the assemblage tend to be both longer and narrower than the flask-shaped forms. The tear-shaped chitinozoans range in length from 48 to 145 μm and have an average length of 96 μm (standard deviation = 20 μm; $N$ = 90) (Fig. 3); they exhibit a length-to-width ratio of about 1.7 : 1. No evidence of coloniality in these forms has been detected. Their vesicles are lanceolate in outline, tapering gradually from the basal region toward the aperture (Fig. 2, A through D, H); they thus lack the flexure that typically separates the body chamber from the neck region in the flask-shaped forms.

The two types of chitinozoans also differ in aperture morphology; in contrast to the hexagonal apertures of the flask-shaped forms, those of the tear-shaped vesicles are circular in outline and tend to be ringed by concentrically arranged bands, flanges, and small, apparently rather regularly spaced bumps (Fig. 2, B through D). Although these Precambrian forms tend to be of relatively small size, they seem generally similar in morphology to younger chitinozoans of the family Conochitinidae (*11, 12*).

These fossils from the late Precambrian Chuar sediments are of interest for the following reasons:

1) To the best of our knowledge, these are the oldest chitinozoans yet discovered; taxa described earlier (approximately 50 genera) are reported to range in age from Early Ordovician (Tremadocian) to Late Devonian (Fammenian), and Chitinozoa have been widely regarded as mid-Paleozoic index fossils (*11, 12*). By extending this range into the late Precambrian, the Chuar microfossils approximately double the known duration of the range zone of the group.

2) The Chuar chitinozoans appear to be among the oldest animal fossils now known. Primarily because Chitinozoa are not represented in the modern biota, the systematic position of the group is uncertain. Chitinozoans are widely regarded as being of animal rather than of plant origin and are considered to have been marine and predominantly planktonic in habit (*12, 14*); most workers have regarded them as ciliate, rhizopodous, or flagellate protozoans (*15*) or as the eggs or egg cases of early metazoans [such as graptolites, gastropods, or polychaete worms (*16*)]. Although supported solely by circumstantial evidence (*12, 16*), the possibility that they are of metazoan affinity cannot be excluded on the basis of currently available data. As for the possibility that they are protozoans, there seems little doubt that heterotrophic, protozoan-like protists, the presumed precursors of the Metazoa, were extant during the late Precambrian. Nevertheless, assured protozoans are as yet unreported from Precambrian sediments (*1–4, 17*). Hence, if the proposed protozoan affinity of the Chitinozoa is correct (and assuming that such microfossils comprise a coherent, natural group), the Chuar microfossils would appear to represent the oldest protozoans now known. Thus, whether of metazoan or of protozoan affinity, the occurrence of abundant chitinozoans in the Chuar sediments would seem to indicate that heterotrophic animals were extant and had apparently become well established at least as early as about 750 ± 100 million years ago.

3) Sediments of the Chuar Group are now known to contain three major as-

semblages of organically preserved microfossils (Fig. 1): the biota preserved in chert lenses in algal laminated carbonates of the Galeros Formation (Carbon Canyon Member) is comprised of cyanophytic filaments and colonial and solitary algal unicells, 2 to 56 μm in diameter (*4, 10*); that of the cherty pisoliths of the Walcott Member of the Kwagunt Formation includes blue-green algal filaments, rare chitinozoans, and spheroidal unicells 10 to about 60 μm in diameter (*8, 10*); and, as mentioned above, the shale biota of the Walcott Member appears to be comprised solely of acritarchs and chitinozoans, the latter ranging to more than 140 μm in maximum dimension (Fig. 3). In addition, shales of the Tanner, Awatubi, and Walcott members of the sequence (Fig. 1) contain the planktonic alga *Chuaria* (*6*), a megasphaeromorphid acritarch that ranges from about 500 to more than 3000 μm in diameter (*18*). Inasmuch as all of these assemblages are of approximately the same geologic age, it seems evident that their compositional differences must largely reflect differences of facies: relatively large planktonic forms, the chitinozoans and *Chuaria*, are predominant in the biotas preserved in the shaley facies whereas the microbiotas of the cherty carbonates (sediments apparently evidencing relatively nearshore, more shallow conditions) are composed chiefly of benthonic algal filaments and small unicells. Although facies-dependent relationships of this type have heretofore been largely overlooked in the Precambrian, their recognition is important; of the various overviews of early evolution set forth in recent years (*2, 4, 19*), virtually all have been based only on those fossils detected in the relatively restricted, shallow-water, cherty carbonate facies. Thus, in light of results summarized here together with important discoveries recently reported from other Precambrian shales (*20*), it seems reasonable to conclude that shaley facies represent a promising, but as yet largely untapped, source of new evidence on the diversity, evolution, and biostratigraphic usefulness of the Precambrian biota.

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## Cellulases Can Enhance β-Glucan Synthesis

Abstract. *β-Glucan synthesis from uridine diphosphoglucose by pea epicotyl tissue slices is increased two- to threefold by preliminary, short-term treatment with cellulases purified from auxin-treated peas. We suggest that cellulases introduce chain ends in accessible regions of cellulose microfibrils which then act as primers for chain elongation.*

Auxin treatment of apical tissue of pea seedlings (*Pisum sativum*, L.) evokes a number of growth reactions (*1*), as well as the simultaneous deposition of large amounts of cellulose (*2*), increased glucan synthetase activity (*3*), and the development of very high levels of two β-1,4-glucanendohydrolases (*4*). The cellulases have been purified to homogeneity and measurements of their physical characteristics (*4*), kinetic properties, and substrate specificities (*5*) indicate that they are distinct proteins which merit designation as true β-1,4-glucan-4-glucanohydrolases (E.C. 3.2.1.4). They hydrolyze linkages in β-1,4-linked glucans, with preference for longer chains and with no detectable activity toward cellobiose, β-glucosides, β-1,3-glucans, or glucan containing alternating β-1,3 and β-1,4 linkages. Transglucosylase activity could not be detected (*5*). Antibodies against the purified enzymes have been used to study their subcellular location and to assay for the appearance and distribution of cellulase messenger RNA (mRNA) (*6*). Pea cellulases appear to be induced by the auxin type of growth regulator, and to be generated and transported in rough endoplasmic reticulum vesicles to the inner surface of the growing primary wall. They may become associated with β-glucan synthetase during this process (*2*). The problem is to assign a function to cellulases in cell walls when rapid net cellulose deposition and extension of the microfibrillar framework are occurring.

Table 1. Preliminary treatment of tissue slices with pea cellulases significantly enhances synthesis of alkali-insoluble glucan. Slices were incubated for 20 minutes, with or without 150 units of pure BS or BI cellulase (*4*) in 0.1 ml of buffer and washed in buffer; glucan synthetase was assayed as described in Fig. 1. Reactions were performed in quintuplicate and incorporation of $^{14}C$ was calculated.

| Preliminary incubation | Glucan synthetase activity* | |
|---|---|---|
| | Alkali-soluble | Alkali-insoluble |
| None (zero time) | 9.81 ± 0.89 | 7.26 ± 0.56 |
| BS (20 minutes) | 11.65 ± 1.07 | 20.56 ± 1.27 |
| BI (20 minutes) | 8.80 ± 0.89 | 13.60 ± 0.84 |
| Buffer (20 minutes) | 9.26 ± 1.67 | 7.59 ± 0.29 |

*Nanomoles glucose incorporated per segment from uridine diphosphoglucose per 10 minutes ± the standard error.