

LACK OF CHITIN IN A SAMPLE OF ORDOVICIAN CHITINOZOA

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ABSTRACT—A sample of *Cyatochitina* sp. (Ordovician Chitinozoa) has been tested for the presence of chitin with the aid of an enzymatic quantitative method based on the use of highly specific chitinases. The fossil remnants of these organisms contain no chitin.

CHITINOZOA, first discovered in 1930 by Eisenack in Silurian deposits from the Baltic, are still enigmatic fossils. Their name (Eisenack, 1931) originates from the external appearance and properties of the body wall which remains unaltered after treatment by concentrated acidic or basic solutions. Nevertheless, the exact chemical nature of the remnants of these organisms has never been elucidated.

We have tested chitinozoa for the presence of chitin with the aid of an enzymatic quantitative method (Jeuniaux, 1963, 1965) using highly specific chitinases purified from cultures of *Streptomyces antibioticus*, following the procedure described by Jeuniaux (1958, 1959). There is relatively little amount of material available in a sufficiently cleaned state and we did not try to analyse the amino acid composition or to search for glucosamine after hydrolysis. Insofar as chitin detection is concerned, the latter method is considerably less specific than the enzymatic one.

The samples analyzed consisted of about 8000 specimens of *Cyatochitina campanulaeformis* Eisenack. This material came from a single glacial Ordovician boulder collected in Mochty (district of Warsaw). The specimens, which had been preserved in glycerin for several years, were washed repeatedly with distilled water. After desiccation under vacuum, the weight of the sample was 6.185 mg.

This material was treated with 0.5 N HCl at room temperature, then with 0.5 N NaOH for 6 hours at 100°C in order to remove protidic components and to bring the eventual chitin to a free state susceptible to hydrolysis by chitinases (Jeuniaux, 1965). After washings with distilled water, the *Cyatochitina* were suspended in a buffer at pH 5.2 (citric acid 0.2 M-Na₂HPO₄ 0.4 M) and pulverized by ultrasonic

waves. The powder was incubated for 8 hours at 37°C with highly purified chitinases (1 ml solution, containing 886 nephelometric units per ml : cf. Jeuniaux, 1958). After incubation, the residual material was centrifuged and the supernatant treated with chitobiase (lobster serum 10 times diluted by distilled water) to hydrolyse the eventual hydrolytic products of chitin into N-acetyl D-glucosamine. The latter substance was then determined by the method of Reissig, Strominger and Leloir, (1955).

We could not detect any trace of acetylglucosamine after the specific treatment by chitinases and chitobiases. The body wall of the *Cyatochitina* studied so far apparently does not contain chitin. The activity of the chitinases and the validity and sensibility of the method cannot be questioned: this method indeed, using the same reagents, has been applied simultaneously to recent *Urmatella* (Endoprocta) cuticles; it revealed the existence of traces of chitin (0.37 gr. per cent) in a sample of 3.76 mg. of dried material. (Bay, Voss-FoucART and Jeuniaux, in preparation).

We can conclude that fossil remnants of *Chitinozoa* do not contain chitin. However, this does not necessarily mean that the body wall of these organisms never contained chitin. The problem of the persistence of chitin through geological time has not yet been resolved although the presence of chitin in a Cambrian fossil, *Hyolithellus*, has been claimed by Carlisle (1964). We are currently studying this problem in our laboratory.

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