CALCIUM FLUORIDE REPLACEMENT TECHNIQUE

A NEW PREPARATION TECHNIQUE FOR ELECTRON MICROSCOPIC STUDY OF NANNOFOSSILS

by

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(with 2 Text-Figures and 2 Plates)

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Because of the small size and microstructure of coccoliths, study with the optical microscope is not always satisfactory. Since the practical limits of resolution of optical microscopes had been achieved by ABBE in the later part of the last century, no basic improvements have been made: the very best lightutilizing microscopes are limited to resolution of objects separated by no less than 1/4 micron, using ordinary light waves.

The work of DEFRANDRÉ and FERT (1952), HALLDAL and MARKALI (1954), BLACK and BARNES (1959) and others not only resulted in the introduction of electron microscopy and the carbon replica technique in the study of nannofossils and nannoplankton but also offers revolutionarily new approaches to the detailed investigation of these organisms.

Carbonate nannofossils, which are opaque to regular electron beams, are replicated or casted by electron beams on a thin transparent film. The greater capacity for magnification of the electron microscope can thereby reveal more details of the structure of nannofossil than had ever been obtained by the optical microscope. However, to some extent difficulties in relating the different results obtained by optical microscopy and electron microscopy must still be overcome.

The carbon replica technique of nannofossils has been eraborated on by various authors such as BLACK (1966), HALLDAL, HAY and TOWE, MCINTYRE (1966) and others. The preparation technique for carbonate nannofossils which has been used successfully by those authors is the so-called preshadow carbon replication method. For details of a revised preshadow replication technique, see HONJO (1967).

Since the stratigraphic significance of nannofossils was first emphasized by BRAMLETTE and RIEDEL in their 1954 paper, several authors have demonstrated the application of nannofossils to age determination and correlation. SULLIVAN's classic works (1964, 1965), and a recent paper by STOVER (1966) are especially note-

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worthy. These authors demonstrated that associations of nannofossils can be used for delimiting biostratigraphic zones.

Application of the electron microscope has been used very successfully for detailed observation of relatively small numbers of fossils. However, all previous biostratigraphic work has employed high-resolution optical microscopies. The tediousness of preparation technique no doubt is one of the main reasons that electron microscopy has not been used for biostratigraphic studies in which a great many samples commonly must be processed. However, the electron microscope is a potentially important biostratigraphic tool given an efficient preparation technique.

Population studies in fossil assemblages has become an important source of information in biostratigraphy. However, quantitative study of nannofossils has not yet been initiated either by optical or electron microscopic methods. Numbers of difficulties are encountered in attempts to make quantitative analyses of nannofossils by electron microscope in addition to the tedium of the preparation.

During an electron microscopic study of chalk samples from Upper Cretaceous deposits of the mid-western part of the United States, we were confronted with unexpected problems in preparation procedures.

Various clay minerals commonly are found in chalk or deep sea oozes. These particles preclude high quality photomicrography, produce aggregation of specimens and sometimes coat individual specimens. Using the preshadow technique, clay particles and specimens are shadowed and coated on the same carbon film and have equal image contrasts under the electron microscope.

Clay particles have been segregated mainly by means of water settling or centrifuging of samples in water in most laboratories. However, inasmuch as small coccoliths are only a few microns across, most of the smaller specimens are decanted away with the clay particles. In addition the larger specimens up to 20 microns in diameter, may be set down much earlier and also discarded.

We observed a marked sorting effect on the assemblage during preparation procedures to remove clay particles and to concentrate into various size and shape fractions. As a result, the final association of specimen on a mesh is usually quite different from the original assemblage.

The technique presented in this paper allows the removal of clay particles and concentrates the nannofossils without changing the original size or shape distribution and is suitable for quantitative analysis using the electron microscope. The clay particles are desolved and the nannofossils, which is usually are composed of calcite, are replaced by calcium fluoride by immersion in hydrofluoric acid. This technique was first suggested by BRAMLETTE and SULLIVAN (1961) for optical study of nannofossils.

Concentrated hydrofluoric acid has been regarded as necessary for efficient removal of clay particles. Although nannofossils appear to be completely replaced

by calcium fluoride under the optical microscope, we found that the surface of specimen is usually corroded replacement occurs. When calcite particles are gently replaced by using dilute acid, this " pre-etching " phenomena is minimized. However, in dilute acid clay particles remain undesolved. MINOURA has found that successive bathings in hydrofluoric acid of different concentrations overcomes this problem.

As a first step, therefore specimens are replaced by calcium fluoride in dilute hydrofluoric acid. Clay particles at this point are not desolved. The sample is then subjected for a prolonged time to concentrated hydrofluroic acid during which all of the remaining clay particles are desolved, while the replaced specimens are not affected. After shadowing and coating, the calcium flouride-replaced specimens are desolved in hydrochloric acid as is done in preparing preshadow replicas. This procedure is described in detail below:

1) Pulverize a piece of sample if necessary.
2) Place approximately 0.5 grams of sample in a polyethylene centrifuging test tube, wet with a small amount of water, and add 5 cc of 15% hydrofluoric acid. Allow to stand for 20 minutes.
3) Centrifuge at a rather high speed for a prolonged time (up to three minutes) until all of the clay fraction is settled. Carefully decant the supernatant.
4) Add 5 cc of concentrated hydrofluoric acid before the sample is dried. Allow to stand for one hour.
5) Centrifuge at high speed for a prolonged period and decant the supernatant as in procedure (3).
6) Add approximately 5 cc of distilled water (triple distilled water is preferable), and centrifuge at high speed until the clay-sized replaced specimens are completely settled. Decant the supernatant.
7) Repeat procedure 6).
8) Heat the test tube for a few minutes in a hot water bath to remove HF (Text-Figure 1)
9) Add a small amount of water to the sample. The sample is then sprayed on a mica substrate (HONJO, 1967). The air blow method (MC INTYRE, 1966, or HONJO 1967) is recommended to produce an even distribution of specimens.
10) Place the mica substrate in a vacuum with specimen side up. The specimens are then shadowed by platinum palladium and coated by carbon. The carbon film is separated from the mica surface by utilizing the surface tension of distilled water. Refer to HONJO (1967) for the details.
11) Transfer the carbon film into 0.5 N hydrochloric acid. A fine stainless steel net is convenient for transfering the fragile film without damaging. Stand film in the acid for 10 minutes.

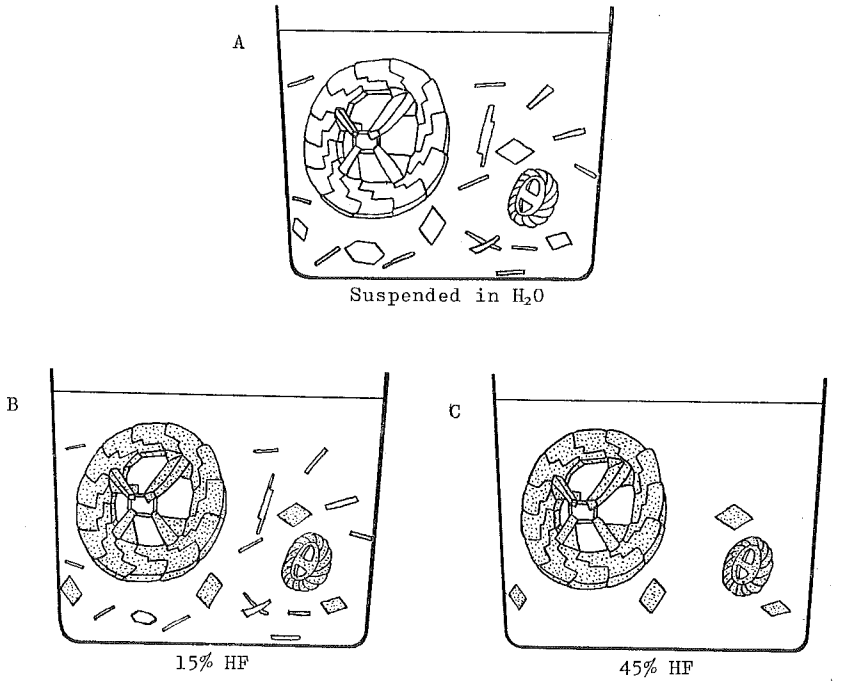

Fig. 1
A chalk or deep sea ooze sample contains small carbonate fragments and aluminosilicate fractions as well (A). Nannofossil specimens with clay minerals are dipped in weak hydrofluoric acid where only carbonate fractions are gently, but completely replaced by calcium fluoride. The majority of clay minerals are remained (B). The whole particles are gathered and re-dipped in concentrated hydrofluoric acid. The remainder of clay particles are desolved and the replaced specimens are collected for study (c).

12) Transfer the carbon film into distilled water. Repeat this rinsing procedure for two times.
13) A piece of carbon film is retrieved from the surface of the water on an electron microscopic mesh and dried. The replica is ready for electron microscopic observation (Text-Figure 2).

The entire carbonate fraction of a piece of Niabrara chalk was concentrated and replicated by this new technique. It was observed that the sample contained a larger number of samll carbonate fragments in various origin than were observed in a replica prepared by the previous technique. We had previously thought that these chalk sample were composed of well preserved coccoliths inasmuch as complete specimens were more commonly encountered than broken ones. However, it appears that a considerable amount of clay-sized carbonate fragments from various sources form an important component of these chalk samples. Also, small

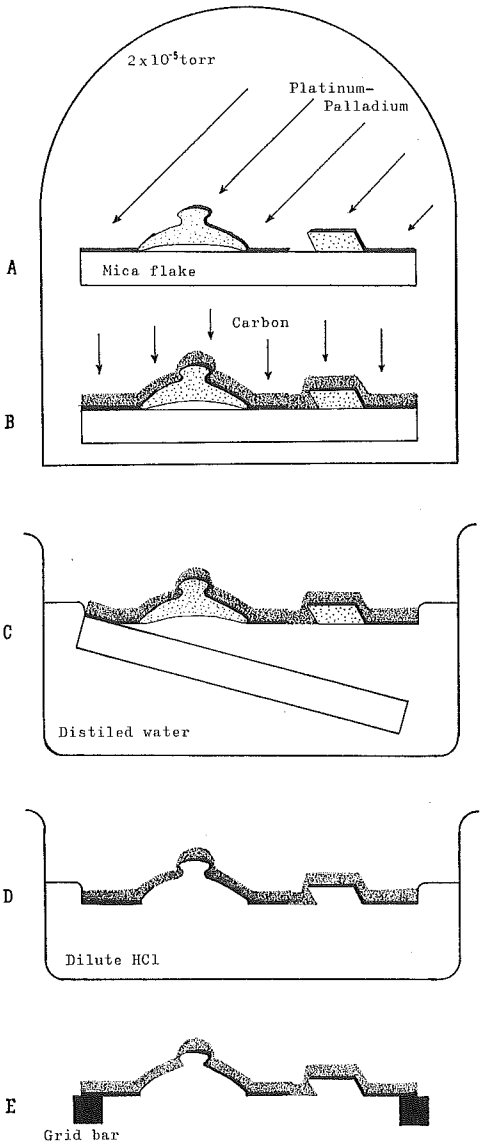

Fig. 2
Calcium fluoride replaced specimens are mounted on a piece of mica flake, then shadowed and carbon coated (A, B). The carbon film is separated from the substrate (C) and calcium fluoride specimens are desolved in hydrochloric acid (B). After rinsing, the carbon film is transferred onto a microscopic mesh (E) for electron microscopic observation.

coccoliths, only a few microns in diameter, are more abundant in the new preparation.

Clay particles, which sometimes fill the tunnels of coccoliths are removed from the surfaces of specimens by this technique. Also, small carbonate particles, which had been difficult to distinguish from clays, can be identified. This technique can be applied samples consisting of nannofossils, clay and other miscelleneous siliceous or calcareous particles as well as to samples in which carbonate particles and nannofossils are of similar size.

Electron photomicrographs of calcium fluoride-replaced preshadowed specimens and specimens preshadowed directly by previous methods are compared in plate 2, figs. 1 to 4. All specimens are from the same source. In replaced specimens, it should be noted that crystalline edges appear slightly beveled and little rounder than those of directly preshadowed specimens.

Our thanks due to HISATAKE OKADA of Hokkaido University for laboratory assistance. We thank Dr. MASAO MINATO and Dr. ANDREW MCINTYRE for suggestions and discussions. Dr. CHARLES ROWETT critically read the manuscript and gave us valuable advices. The Hitachi 11B electron microscope was used throughout this research. Our ultramicroscopic study of nannofossils is supported by Grant-in-Aid A-091008 from the Japanese Ministry of Education.

References

BLACK, M. (1965): Coccoliths, Endeavour, vol. 24, no. 93, p. 137.

BLACK, M. and BARNES, B. (1959): The structure of coccoliths from the English Chalk, Geol. Mag., vol. 96, no. 5, pp. 321–328, pls. 8–12.

BRAMLETTE, M. N. and RIEDEL, W. R. (1954): Stratigraphic value of discoasters and some other microfossils related to recent Coccolithophores, Jour. Paleont., vol. 28, no. 4, pp. 385–403.

BRAMLETTE, M. N. and SULLIVAN, F. R. (1961): Coccolithophorids and related nannoplankton of the early Tertiary in California, Micropaleontology, vol. 7, no. 2, pp. 129–188.

DEFLANDRE, G. and FERT, C. (1952): Sur la structure fine de quelques coccolithes fossiles observés au microscope électronique, signification morphogénétique et application a la systématique, Acad. Sci., C.R., vol. 234, pp. 2100–2102.

EDWARDS, A. R. (1963): A preparation technique for calcareous nannoplankton, Micropaleontology, vol. 9, no. 1, pp. 103–104.

HALLDAL, P. and MARKALI, J. (1954): Observation on coccoliths of *Syracosphaera mediterranea* Lohmann, *S. pulchra* Lohmann, in the electron microscope, Jour. Cons. Internat. pour l'explor. de la Mer., vol. 19, no. 3, pp. 329–336.

HAY, W. W. and TOWE, K. M. (1962): Electron microscopic examination of some coccoliths from Donzacq (France), Eclogae Geol. Helv., vol. 55, no. 2, pp. 497–518, pls. 1–10.

HONJO, S. (1967): Electron microscopic techniques in carbonate geology and nannofossil study, Rep. Res. Kansas Geol. Sur., (to be published)

HONJO, S. and BERGGREN, W. A. (1967): A study of planktonic foraminifera by scanning electron microscope, Micropaleontology, vol. 13, (to be published)

MCINTYRE, A. (1966): Oral presentation to the GSA annual meeting, 1966.

STOVER, L. E. (1966): Cretaceous coccoliths from France and the Netherlands, Micropaleontology, vol. 12, no. 2, pp. 133–167.

SULLIVAN, F. R. (1964, 1965): Lower Tertiary nannoplanktons from the California Coast Ranges, I: Paleocene and II: Eocene, Univ. Calif. Publ. Geol. Sci., vol. 44 and 53, pp. 163–228 and pp. 1–52.

(Manuscript received January 25, 1967)

PLATE 49 AND EXPLANATION

Explanation of Plate 49

The wide-field electron photomicrograph (figure 1) shows the carbonate fraction of a piece of Niabrara chalk which is concentrated and replicated by the calcium fluoride replacing technique. It is observed that the sample contains a large number of samll carbonate fragments in various origin, than are observed in a replica prepared by the previous technique. Hitachi wide-field pole-piece was used for the photography.

Undescribed coccolith with a typical size (figure 2) and a small coccolith (figure 4) which is usually missed during preparation procedure, are compared.

A specimen which is prepared by a single bathing in concentrated hydrofluoric acid is illustrated in figure 3. Note on a goose-skined "pre-etched" surface of the specimen.

Plate 49

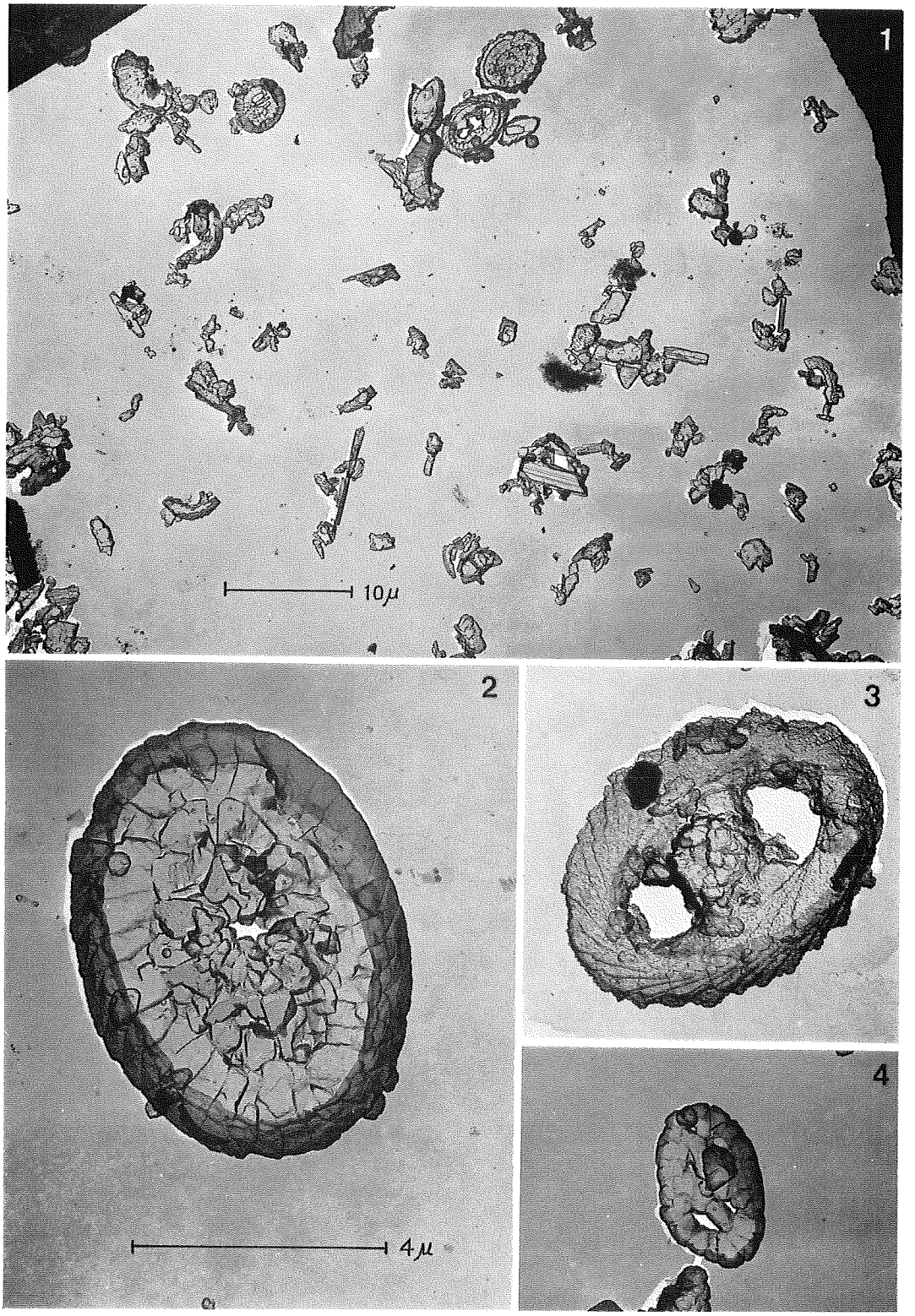

PLATE 50 AND EXPLANATION

Explanation of Plate 50

Electron photomicrographs of calcium fluoride replaced, preshadowed specimens (figure 1, 3) and specimens preshadowed directly by previous methods (figure 2, 4), are compared. All specimens are from the same source. Figure 1, 2: Undescribed species of *Cretadiscus*. Figure 3, 4: *Chiastozygus diplogammus* (Deflandre).

Plate 50