

NEW METHODS IN PALEOBOTANICAL MICRO-TECHNIQUE

BY

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The old method of examining a coal ball for interesting plant material which it might contain was to quarter it with a rotary diamond saw and then to prepare thin sections along the horizontal and vertical cuts. These thin sections had to be sliced off with the diamond saw and ground with at least three different grades of carborundum powder until microscopic thinness was obtained. The grinding was done either on glass plates or on rotating laps driven by motor power. To produce a good thin section mounted on a glass plate with Canada balsam took approximately one and a half to two hours; it took about one day to prepare a single coal ball for the preliminary tests.

Since 1928 when Walton and Koopmans published their cellulose peel method great progress has been made in the testing of coal balls in my laboratory. If the coal ball is sufficiently hard and not too porous all adherent coal and other mineral matter is removed with a hammer and the coal ball is dipped into a 10 per cent solution of hydrochloric acid. The latter is allowed to remove the surface of the uncut coal ball until any enclosed plant material becomes visible. Usually stem or root pieces, possibly also seeds which touch the surface can be seen and the orientation of the cuts can be planned. In accordance with the surface indications, the coal ball is now cut with the diamond saw into two, four or more blocks. The next step is to smooth the cut surfaces with carborundum powders in the successive grades of 120, 220, and 600, treating with a 10 per cent solution of hydrochloric acid until the plant organs are clearly visible. The cuts are carefully washed with water and dried. Their surfaces now present a very fine velvet of carbonized plant tissues from which, to a depth of perhaps 50 to 100 micra the matrix of calcium carbonate has been removed. This velvet is now soaked in butyl acetate and a 10 per cent solution of nitrocellulose in butyl acetate is poured over the moist surface. The use of nitrocellulose dissolved in butyl acetate has been worked out by one of my students, Roy Graham, who published his method in *Stain Technology*, April, 1933, pp. 65-68. The nitrocellulose solution is most satisfactory. We have tried solutions of parlodion or of pyroxilin in ether and alcohol, or of cellulose acetate in acetone, or of purified collodion. The nitrocellulose solution produces a satisfactory film which does not curl but sticks closely to the surface of the coal ball until removed with a safety razor blade. It also gives a very transparent film which does not need to be cleared with xylol or with Eycleshymer's clearing fluid. It is usually necessary to apply several coats of nitrocellulose. The successive coats can be applied as soon as the previous coat begins to harden. I found that three coats are usually necessary to produce a very coherent film. The films can be filed in paper envelopes to be examined at any convenient time. For the examination I use a binocular microscope with a magnification of about 25 or 50. If the film discloses interesting details, squares are cut out of it with a safety razor blade and mounted on glass slides in Canada balsam. The drying of a film takes 6 to 8 hours but it is easy to prepare a dozen coal balls in a day and have the films dry during the night. Nitrocellulose solutions in butyl acetate are not very sensitive to moisture and sufficient drying of etched coal balls can be accomplished in an electric oven in a few minutes. The new method is at least ten times faster than the old one. The same advantages prevail when it comes to the minute study of selected material for research purposes.