which is flexible enough to allow for growth of the stems. Plastic tubing may be used to set up a drainage system for the tote box. In order to simulate soil conditions and provide better support for the root system of the plant, plastic beads or debris may be used in the bottom of the box.

The expanding plastics industry has placed an infinite number of containers, dispensers, tubing, and analytical equipment on the market. These items can easily be adapted for trace element studies. Plastics, indeed, can provide an answer to the ever-present question of expense and practicality.

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Microradiography of Microfossils with X-Ray Diffraction Equipment[1]

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Several articles have appeared recently presenting different techniques of microradiography and showing their value in the study of thin sections, primarily of biological and medical material (*1, 2*). This method is valuable in the study of whole specimens as well as thin sections, as will be shown by the results with paleontological material (*3*).

Calcareous, siliceous, and pyritic microfossils of varying size and thickness were radiographed with x-ray diffraction equipment. The internal arrangement of foraminiferal chambers and of muscle-scar patterns of Ostracoda, and the presence or absence of pores in shells of both groups were detected where preservation was favorable—sometimes when no other method had been successful. Furthermore, no harm was done to the specimens.

The type of x-ray diffraction apparatus used is available in crystallographic laboratories and mineralogical departments of many institutions. This apparatus usually has 4 windows through which x-rays are transmitted into special cameras. The removal of one of these cameras makes it possible to utilize the x-rays for microradiography without any modification of the apparatus itself. The radiation is directed approximately horizontally in the equipment used (North American Philips), so that it is necessary to mount specimens and photographic plate vertically. A simple stand can be used, placing the glass photographic plate in a holder, with the specimen supported in front of it. Precautions must be taken to guard the operator from exposure to x-rays.

Diffraction apparatus has interchangeable tubes with targets of any one of several metals, permitting essentially monochromatic radiation of any one of several wavelengths between 0.6 and 2.3 A. The radiation in all cases is long wavelength, or soft, which is favorable for detecting the thin delicate structures that would not be seen if short or hard rays were used. No filter was used in this work.

[1] Publication authorized by the Director, U. S. Geological Survey.

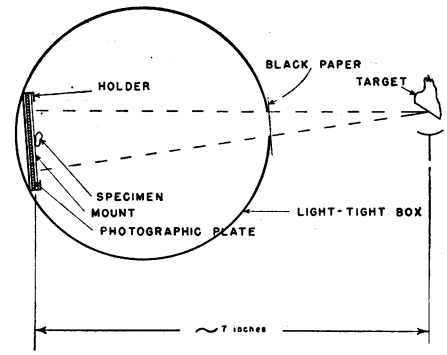

FIG. 1. Diagrammatic sketch of holder.

For microfossil specimens averaging about 0.5 mm or less in thickness, the factors, with a copper target tube, were 25–35 kv (rarely 45 kv), 15–20 ma, and 5–20 min. Time and voltage are the variables, the values depending on the size and chemical composition of the material. Distance from the target to the film is approximately 7 in., which gives a large enough beam of x-rays to cover the specimens.

To support the specimens and photographic plate, a diffraction camera was adapted by fitting a holder for the plate and specimen in the camera, opposite an opening transparent to x-rays (Fig. 1). Any light-tight box with such an opening may be used. The opening is placed directly against the x-ray window. This setup permits close contact between specimen and emulsion, thus lessening blurring (*4*). It is also easy to center the setup rapidly for each exposure.

The microfossils are mounted with gum tragacanth on cleared x-ray film, both of which are transparent to x-rays. The cleared film provides a firm support for the specimens, which can easily be placed in any position for microradiography. Eastman 548-O spectroscopic plates (an extremely fine-grained emulsion)

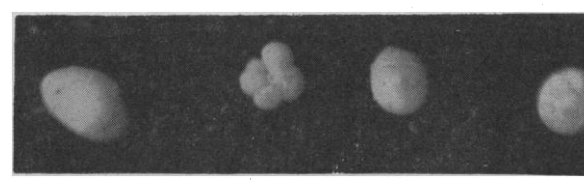

FIG. 2. Photograph of group of Foraminifera. Approx. × 36.

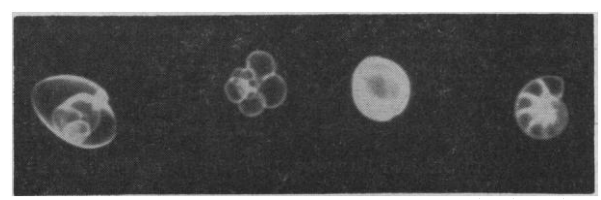

FIG. 3. Microradiograph of same Foraminifera, showing arrangement of chambers. Approx. × 36.

were used. These glass photographic places are cut to fit the holder, and the mounted specimen and plate are placed in the holder in the darkroom. The photographic plates were developed in D-11, D-19, or DK-50. For the maximum enlargement desired (less than 100 times) all these developers were satisfactory.

Although stereoscopic microradiographs have not been made by the author, they could easily be taken, particularly if the setup permits mounting the specimens horizontally rather than vertically (*5*).

Figs. 2 and 3 are, respectively, a photograph and a microradiograph of a group of Foraminifera. The internal arrangement of chambers is plainly visible in the microradiograph.

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The Use of an Ion Exchange Resin for the Hydrolysis of Casein and Coffee Proteins

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A study of the noncaffeine nitrogenous constituents of coffee has led to an investigation of the amino acids present in coffee proteins. The method used initially was the classical hydrochloric acid hydrolysis followed by chromatographic separation of the amino acids on a column of 200–400 mesh Dowex-50,[2] patterned after the technique of Moore and Stein (*1*). Since there was the customary humin formation during hydrolysis, other methods were sought.

It has been observed (*2, 3*) that insoluble cation exchange resins in the hydrogen form act as catalysts for certain hydrolytic reactions, such as ester hydrolysis and sucrose inversion. Dowex-50, a sulfonated styrene resin, behaves as an insoluble strong acid in aqueous solutions, and it was believed that it might furnish sufficient hydrogen ions to accomplish the hydrolysis of proteins. The fact that the resin removes most of the amino acids from the solution as they are formed would also be expected to favor the hydrolysis. Casein was selected for exploratory experiments. Accordingly, 3 g casein, 15 g 200–400 mesh Dowex-50, and 300 ml water were mixed thoroughly and refluxed. The solution gave a negative biuret reaction after about 70 hr. Boiling was continued for an additional 24 hr to insure completion of the reaction. At the end of the hydrolysis, most of the amino acids were on the resin. By using barium hydroxide, all the amino acids were stripped from the resin. This barium hydroxide extract was then treated with sulfuric acid to remove the barium and to give essentially a solution of amino acids. The amino acids also may be fractionally removed by use of HCl or various buffers. The same procedure was found satisfactory for the hydrolysis of proteins from a sample of green Santos coffee.

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[2] Obtained from the Dow Chemical Company, Midland, Mich.

TABLE 1

ANALYSES OF COFFEE PROTEIN HYDROLYSATES PREPARED USING HYDROCHLORIC ACID AND ION EXCHANGE RESIN

	Milligrams of nitrogen	
	HCl hydrolysate	Resin hydrolysate
Alanine	2.86	2.74
Aspartic acid	5.12	4.93
Glutamic acid	8.74	1.97
Leucine	5.58	5.44
Phenylalanine	2.06	2.07
Serine	1.56	1.46
Threonine	0.95	0.94
Valine	2.57	2.39
Basic amino acids and humin	15.39	14.70
Ammonia	4.89	4.50
Total recovered	54.25	45.52
Total in hydrolysate	61.60	61.60

The protein fraction of the coffee was prepared by water extraction of the ground green coffee. HCl was added to precipitate the protein. The precipitate was washed with water, then alcohol and ether, and finally dried. The HCl hydrolysate of this protein was prepared by refluxing 400 mg protein with 8 ml 6 *N* HCl on an oil bath at 135° C for 12 hr. After hydrolysis, the excess HCl was removed by distillation *in vacuo*.

The Dowex-50 hydrolysate was prepared from 400 mg of the protein by the method indicated above, using 40 ml water and 2 g of the resin. The amino acids were removed from the resin by the use of barium hydroxide. This hydrolysate was nearly clear in contrast to the dark, humin-containing solution obtained by carrying out the hydrolysis with HCl. It has been suggested (*1*) that the amount of humin produced in an acid hydrolysate of a protein can be held to a minimum by use of a large excess of acid. To be certain that the low humin formation in the resin hydrolysate was not due to the excess of liquid phase present, another portion of the protein was hydrolyzed for 12 hr with 200 times its weight of 6 *N* HCl. This procedure did appear to decrease humin formation somewhat, but the hydrolysate still became very dark and some insoluble humin was produced.

Both the HCl and resin hydrolysates were chromatographed on Dowex-50, using a column 110×1.5 cm. The amino acids were fractionally eluted from the column, using acetate buffers. The buffers were put through the column by the use of nitrogen gas under a pressure of 200 mm Hg. The individual fractions