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

were analyzed by measuring the intensity of the blue color produced upon heating with ninhydrin solution (*4*). Ammonia nitrogen was determined by the classical aeration procedure. The use of barium hydroxide for removing the amino acids from the resin would probably cause large losses of any ammonia present; therefore, the ammonia nitrogen of the resin hydrolysate was determined by aerating a hydrolysate in which the amino acids were removed from the resin by use of hydrochloric acid rather than barium hydroxide. Basic amino acids plus humin were determined by phosphotungstic acid precipitation followed by Kjeldahl determination for nitrogen.

The analyses of the two hydrolysates are shown in Table 1.

By using the method of Graham *et al.* (*5*), the tryptophan content of the coffee protein was found to be 1.7%, thus accounting for an additional 0.93 mg of nitrogen in Table 1. Tryptophan is destroyed by acid hydrolysis or by refluxing with distilled water; hence it was not found in either hydrolysate. No hydroxy proline was found using the method of Newman and Logan (*6*). Using paper chromatography techniques, only a trace of tyrosine was detected, and arginine, histidine, and lysine were shown to be present.

It may be observed from Table 1 that ammonia nitrogen is slightly higher for the HCl hydrolysate, indicating perhaps more degradation or deamination in this procedure. Furthermore, a notably low glutamic acid value was obtained in the resin hydrolysate, and some of the others tend to be slightly low when compared with the HCl hydrolysate. It is probable that the glutamic acid in the resin hydrolysate formed pyrrolidone carboxylic acid (*7*). This acid might tend to form salts with the other amino acids and thus interfere with the chromatographic separation. That the low value of glutamic acid in the resin hydrolysate was due to pyrrolidone carboxylic acid formation is borne out by the fact that upon treatment with HCl, glutamic acid appeared in the resin hydrolysates in an amount comparable to that found in the original HCl hydrolysate.

Using commercially available vitamin-free casein, resin hydrolysates have been prepared by the above method. On evaporation, a crystalline, almost white product is obtained. This material readily dissolves in water and will serve as the sole source of nutrients for many microorganisms at 0.5% level. The resin hydrolysis of casein may be hastened by the use of a small amount of HCl. For example, 6 g casein, 30 g Dowex-50, and 600 ml 0.05 *N* HCl, or 60 g casein, 300 g Dowex-50, and 1700 ml 0.1 *N* HCl, show no biuret reaction after 48 hr.

The method of hydrolyzing proteins by the use of ion exchange resins shows considerable promise as a technique in the study of proteins. Recent studies using casein have shown that there may be some simple peptides present in the resin hydrolysates; however, indications are that the method gives nearly complete hydrolysis of the proteins studied. Work is continuing on the characterization of other proteins and the optimum conditions for carrying out hydrolyses using Dowex-50 and other resins.

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Comments and Communications

Teacher Training

IN THE United States as a whole far too little attention is given to training teachers of science. This is true of the teachers in the grades who must conduct science study periods, of "general science" teachers in junior high schools, and of teachers of physical and biological science in high schools.

This communication is not a plea for more national committees to prepare more excellent reports, which will be studied by too few of the right people and which will probably be acted on by almost no one; rather, it is a plea for the scientists in each college and university to act now. Fortunately, there are appropriate actions that can be taken and that will not entail great expense.

Relatively few of our colleges and universities have adequate teacher-training programs that provide *suitable* courses in science. The usual beginning course in a natural science is almost certain to be aimed at the production of more specialists in that science. To get a broad training in science, the prospective teacher would have to complete at least 30–50 semester hours of such first-year college courses. In addition, the teacher of senior high-school science should specialize in the particular science he or she plans to teach.

To expect such extensive training of our public school teachers is entirely unrealistic. The relatively low salary scales have attracted an entirely inadequate supply of students into teacher-training programs. The immediate problem that needs our attention is how to provide training in science for students who are now preparing to become grade and high-school teachers. This problem may be broken down into two