bide[4] and decaborane.[5] The linked icosahedra in boron and boron carbide give strong network structures in which surprisingly large holes are interspersed among the very compact icosahedral groups.

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RECEIVED MARCH 5, 1951

(4) H. K. Clark and J. L. Hoard, THIS JOURNAL, **65**, 2115 (1943).
(5) J. S. Kasper, C. M. Lucht and D. Harker, *Acta Cryst.*, **3**, 436 (1950).
(6) Du Pont Post-Doctoral Fellow, 1949–1950.

CHROMATOGRAPHY OF PROTEINS. RIBONUCLEASE

Sir:

It has been demonstrated that elution analysis is capable of extremely high resolving power when either starch columns[1] or ion exchange resins[2] are used for the fractionation of mixtures of amino acids. The advantages of bringing proteins within the scope of such chromatographic methods are too obvious to require elaboration. After many unsuccessful attempts, employing several different proteins and numerous adsorbents, satisfactory chromatograms have been achieved with ribonuclease and with lysozyme on columns of the carboxylic acid resin IRC-50. A finely ground preparation of the resin (XE-64, 250 to 500 mesh) was employed, for which we are greatly indebted to Dr. James C. Winters of The Rohm and Haas Company. It should be mentioned that Paléus and Neilands[3] have employed columns of IRC-50 for the purification of cytochrome C.

The results obtained with ribonuclease are given in Curve A of Fig. 1. About 3 mg. of crystalline ribonuclease (assaying 44 units per mg.), kindly supplied by Dr. M. Kunitz, was chromatographed. The protein concentration (solid circles) in the effluent was measured by the photometric ninhydrin method[4] and the ribonuclease activity (open circles) by the spectrophotometric method of Kunitz.[5] It can be seen that about three-fourths of the ninhydrin positive material emerges as a sharp peak around 18 effluent cc. Ahead of this peak, however, beginning at about the column volume (6 cc.), approximately one-fourth of the material appears as a spread out zone. The position of the main peak may be changed at will by variations in the *p*H of the eluting buffer. With phosphate buffers of lower *p*H the protein travels more slowly; with a higher *p*H, more rapidly.

It is of interest that ribonuclease emerges from the column with its enzyme activity undiminished, and further, that the amount of activity and the ninhydrin color value parallel one another very closely. If the material in the two peak fractions of Curve A is pooled and rechromatographed, a single symmetrical peak (Curve B) is obtained.

(1) S. Moore and W. H. Stein, *J. Biol. Chem.*, **178**, 53 (1949).
(2) W. H. Stein and S. Moore, *Cold Spring Harbor Symposia on Quant. Biol.*, **14**, 179 (1950); S. Moore and W. H. Stein, to be published.
(3) S. Paléus and J. B. Neilands, *Acta chem. Scand.*, **4**, 1024 (1950).
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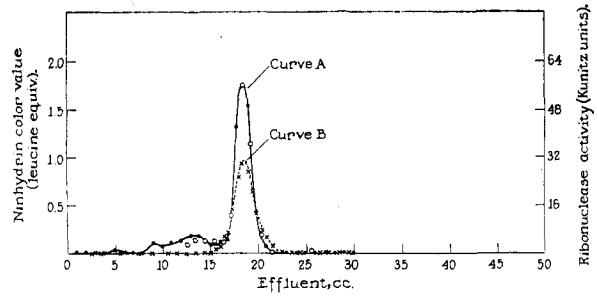

Fig. 1.—Chromatography of ribonuclease. The column of IRC-50 was 0.9 × 30 cm. Elution was performed with a 0.2 *M* sodium phosphate buffer of *p*H 6.45 at a rate of 1 to 1.5 cc. per hour. The effluent was collected in 0.5-cc. fractions. Curve A, crystalline ribonuclease; ●—● ninhydrin color; O—O ribonuclease activity. Curve B, rechromatography of material from peak of Curve A; ×—×, ninhydrin color.

Although the results obtained thus far suggest inhomogeneity, it will be necessary to isolate and characterize the material from various parts of Curve A before it can be concluded with certainty that the sample of ribonuclease employed in these experiments is impure.

When crystalline lysozyme carbonate was chromatographed on a column similar to the one described in Fig. 1, the protein appeared in the effluent as two well-separated symmetrical peaks at about 25 cc. and 40 cc. Elution was performed with a 0.2 *M* phosphate buffer of *p*H 7.2. The sample of lysozyme carbonate was kindly supplied by Dr. C. A. Stetson, Jr. Experiments of Dr. Harris H. Tallan, which will form the basis of a subsequent communication, have indicated that lytic activity resides in both protein peaks.

Because success has attended the chromatography of relatively stable low molecular weight proteins, it should not be inferred that the same experimental conditions will prove applicable to numerous other more fragile proteins. The present results do indicate, however, that substances with a molecular weight as high as 15,000 to 20,000 distribute themselves sufficiently rapidly between a solution and an ion exchange resin to permit near equilibrium conditions to be attained in a chromatogram flowing at an appreciable rate.

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RELATION OF MOVEMENT TO TIME IN IONOGRAPHY

Sir:

In recent reports on ionography, that is, the migration of ions or charged particles on paper in an electric field, it has been reported that the movement of certain substances was not a linear function of time.[1,2] However, it has been found possible, under proper conditions, to obtain the same mobility, *i. e.*, the migration computed on the basis of cm./sec. per volt/cm., for relatively long periods of time. This indicates a linearity of movement with

(1) E. L. Durrum, THIS JOURNAL, **72**, 2943 (1950).
(2) E. L. Durrum, *Science*, **113**, 66 (1951).