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.

BAKER LABORATORY OF CHEMISTRY J. L. HOARD
CORNELL UNIVERSITY S. GELLER[6]
ITHACA, NEW YORK R. E. HUGHES

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).
(4) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).
(5) M. Kunitz, *ibid.*, **164**, 563 (1946).

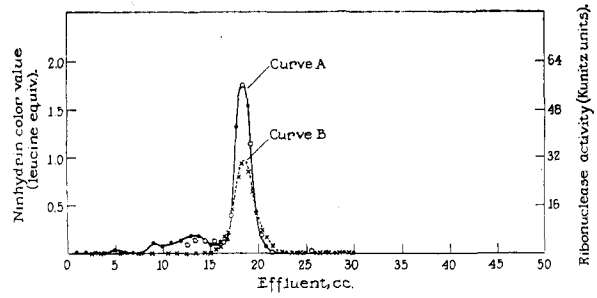


Fig. 1.—Chromatography of ribonuclease. The column of IRC-50 was 0.9 × 30 cm. Elution was performed with a 0.2 *M* sodium phosphate buffer of *p*H 6.45 at a rate of 1 to 1.5 cc. per hour. The effluent was collected in 0.5-cc. fractions. Curve A, crystalline ribonuclease; ●—● ninhydrin color; O—O ribonuclease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

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH C. H. W. HIRS
NEW YORK 21, N. Y. WILLIAM H. STEIN
STANFORD MOORE

RECEIVED MARCH 16, 1951

## 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).

respect to time. The establishment of the conditions for obtaining this relationship is a critical factor in the development of the quantitative aspects of this technique. Fig. 1 shows the results obtained with leucine, phenylalanine and pepsin.

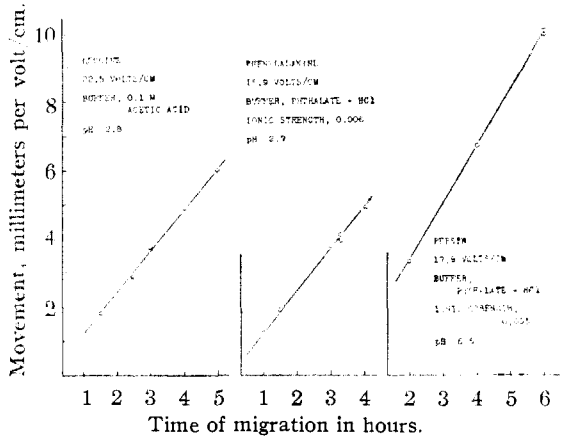


Fig. 1.—Movement of compounds on paper, in an electric field, as a function of time: pepsin, crystallized, of porcine origin, Armour Labs.; temperature, 23–24°.

The experiments were carried out in a modification of the apparatus previously described.[3,4,5] Six paper strips (E. & D. No. 613, 8 mm. wide) stretched across a Bakelite frame, were held in place by plastic draw-bolts actuated by stainless steel coil springs. At each end of the frame, the ends of the strips dipped into common buffer vessels, each containing about one liter of buffer solution. The frame, paper strips and buffer vessels were completely enclosed within a vapor-tight Lucite chamber. The vapor space in the chamber was kept to a minimum by replacing as much of the air as possible with water. Electrical contact with the buffer solutions was made by agar–KCl salt bridges.

The dry paper strips were placed in the Bakelite frame and wetted by immersing the frame in the buffer solution. The excess liquid was allowed to run off, as indicated by the absence of sheen on the paper; the frame was then placed in position in the chamber. When the reading of the milliammeter, in series with the paper strips, became relatively constant, 0.01 ml. of the substance under study (2–8 mg. per ml.) was added to the center point of each horizontal paper strip. The *p*H of the buffers was checked before and after each run to insure against variation. Ninhydrin was used to identify the amino acids, and lead acetate–bromo phenol blue for the pepsin. The displacement of the forward edge of the band on the paper, from the initial point of application of the migrating substance, was measured in each case.

DEPARTMENT OF BIOCHEMISTRY
THE GRADUATE SCHOOL AND
STRITCH SCHOOL OF MEDICINE
LOYOLA UNIVERSITY
CHICAGO 12, ILL.

HUGH J. MCDONALD
MATTHEW C. URBIN
MARTIN B. WILLIAMSON

RECEIVED FEBRUARY 10, 1950

(3) H. J. McDonald, M. C. Urbin and M. B. Williamson, *Science*, **112**, 227 (1950).

(4) H. J. McDonald, M. C. Urbin, and M. B. Williamson, Absts., Div. Biol. Chem., 118th Meeting, Amer. Chem. Soc., p. 66C (1950).

(5) H. J. McDonald, M. C. Urbin and M. B. Williamson, *J. Colloid Sci.*, in press.

## THE EXCHANGE REACTION BETWEEN EUROPIUM(II) AND EUROPIUM(III) IONS IN HYDROCHLORIC AND PERCHLORIC ACID SOLUTIONS

*Sir:*

In view of current interest in electron transfer exchange reactions, we make this preliminary report of our studies of the measurable exchange rate between europium(II) and europium(III) ions in aqueous solutions of hydrochloric and perchloric acid.

Solutions of europium(II) chloride (perchlorate) and labelled europium(III) chloride (perchlorate) were mixed in the appropriate acid, aliquots removed at definite time intervals, the two oxidation states separated, and the specific activity of the europium(II) fraction determined by counting the solution in a reproducible geometry with a dipping Geiger-Mueller tube, then precipitating the europium and weighing it as $Eu_2(C_2O_4)_3 \cdot 10H_2O$. Sodium chloride or sodium perchlorate was used, as required, to adjust the ionic strength to 2.0 in all runs. Since europium(II) in aqueous solution is easily oxidized by air, and is known[1] to be photochemically oxidized by water, all experiments were carried out in an opaque reaction vessel under nitrogen freed from oxygen by passage through a chromium(II) chloride solution. Runs were made at 39.4 ± 0.1°.

Several chemical separation methods were tried, of which only the precipitation of europium(III) hydroxide from the solution by ammonium hydroxide gave reproducible results. This method led to a small, reproducible induced exchange (approximately 12%).

The tracer used was 5.2-year $Eu^{152}$ produced by the Eu($n$, $\gamma$) reaction in the Oak Ridge pile.

All runs exhibited the usual exponential time dependence of exchange extent. In perchlorate medium the half-time of exchange was not directly measurable since the europium(II) was completely oxidized by the perchlorate ion before the exchange had proceeded more than a few per cent. However, the exchange rate was found to be much faster in the presence of chloride ion, and experiments showed that the rate constant is proportional to the first power of the chloride ion concentration (varied by substituting perchlorate ion for chloride ion). In the presence of chloride the perchlorate oxidation of europium(II) was not serious since the exchange reaction was considerably faster than the oxidation. A plot of rate constant *vs.* chloride ion concentration gives a straight line extrapolating through zero rate at zero chloride ion concentration, implying that the rate of exchange in perchlorate medium in the absence of chloride is very small relative to the rate in the presence of chloride.

The change in half-time as a function of the europium concentrations showed that the reaction is first order in europium(II) and first order in europium(III). The rate is essentially independent of hydrogen ion concentration in the range 0.3–1.0 *f*, indicating that hydrolyzed species are apparently unimportant in the exchange. The exchange rate, $R$, is then given by $R = k\,[\mathrm{Eu(II)}][\mathrm{Eu(III)}][\mathrm{Cl^-}]$. Some representative data, including the termolecular rate constant $k$, are given in Table I.

(1) D. L. Douglas and D. M. Yost, *J. Chem. Phys.*, **17**, 1345 (1949).