[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

The Ion Exchange Behavior of Some Neutral Amino Acids[1]

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Equilibria have been measured in (a) the exchange of amino acid cations for hydrogen ions on two sulfonic acid resins, (b) the exchange of amino acid anions for chloride ions on a quaternary amine resin, (c) the binding of amino acid dipolar ions by the hydrogen form of the sulfonic acid resins. The affinity of the amino acids for a resin has been found to increase with the size of the side chain of the amino acid, but is notably depressed by the introduction of a hydroxyl group. In general, the relative affinities of the three resins are similar although some specific differences have been observed. From the equilibrium data exchange constants have been calculated for seven amino acids and theoretical retardation volumes have been computed. Elution chromatograms of proline and hydroxyproline are reported to illustrate the relation between (a) exchange constants and retardation volumes, and (b) retardation volumes and the ratio of solute load to the capacity of the column.

Because amino acids can exist both as anions and as cations the use of ion exchange procedures for the analysis of mixtures of these compounds offers a variety of possibilities. With the proper choice of exchange agent and with appropriate control of *p*H, it is a simple matter to isolate the acidic, basic and neutral amino acids as separate groups. That the individual members of a group of amino acids of the same charge type can also be separated from one another has been demonstrated experimentally by Stein and Moore,[2] Partridge,[3] and others. The separations accomplished by these investigators depended in some degree on differences in ionization constants but were undoubtedly due primarily to differences in intrinsic affinities of the cations of the amino acids for the ion exchange agents which were employed.[4]

The present paper records measurements of the exchange equilibria of a representative group of neutral amino acids with two cation exchangers and with one anion exchanger. These studies were undertaken as a basis for the rational use of ion exchange columns in the chromatography of amino acids. A few chromatograms of proline and hydroxyproline are also reported. These provide data for testing the validity of theoretical relations between exchange equilibria and retardation volumes under a range of conditions of load and of flow rate.

Equilibrium Studies

Equations for Exchange Equilibria.—Consider the reaction RX + Y = RY + X, where R represents a single ion-combining site on an exchanger and X and Y represent a pair of exchangeable univalent ions. It will be assumed that all binding sites are equivalent and that interaction between them is negligible. In this situation the Law of Mass Action may be applied as though R represents a univalent reactant.

Let m g. of exchanger, in the form RX, be added to 1 liter of a solution containing only Y, or a mixture of X and Y, and the equivalent amount of a counter ion. Let T_R be the equivalents of R in 1 g. of the exchanger, T_C be the concentration of counter ion in the solution, A, B, be the respective numbers of equivalents of X and Y bound to m g. of the exchanger at equilibrium, a_0, b_0 be the initial concentrations in solution, and a,b, the equilibrium concentrations of X and Y, respectively.

The Mass Law defines an exchange constant

$$K = aB/bA \qquad (1)$$

such that K is a measure of the tendency for Y to replace X on the exchanger.

Equation 1 may be transformed into the useful forms

$$B = \frac{mT_RKb/a}{1 + K\frac{b}{a}} = \frac{mT_RK\frac{b}{T_C}}{1 + (K-1)\frac{b}{T_C}} \qquad (2a, 2b)$$

These two equations expose the formal identity of the Mass Law expression with the equation for a Langmuir adsorption isotherm. For the special case of an exchange adsorption it will be seen (equation 2a) that the extent of adsorption of Y is a function, not of the concentration of Y in the solution, but of the ratio of the concentrations of the two competing ions. Equation 2b leads to the alternative generalization that the fraction of the total binding sites which are neutralized by Y is a function of the fraction of the total counter ions in solution which are neutralized by Y. It is also interesting to note (2b) that the conventional isotherm relating B to b is linear when $K = 1$. When K is greater than 1, the curve is convex to the B-axis. It is concave to this axis when $K < 1$. These distinctions are significant to the chromatographic behavior of ions in exchange columns. If, for example, X were the ion employed to develop a chromatogram of a group of other ions, those having K-values greater than unity would give a typical elution chromatogram in which each ion would advance at a rate defined by the value of its own K. Those ions for which K was less than unity would tend to develop as a displacement chromatogram in which the bands would be contiguous and would move at the rate of the boundary of the displacing ion.

In the absence of other ions the following relations must obtain: $a = T_C - b$, $B = a - a_0 = b_0 - b$. Since T_C, m, a_0 and b_0 are known from the composition of the original reaction system and, since T_R may be determined in independent experiments, a determination of b in any equilibrium system suffices to permit computation of K from equations 2a or 2b. Actually T_R as well as K may be derived by the graphic analysis of a series

(1) This work was supported in part by a grant from The Rohm & Haas Company, Resinous Products Division, Philadelphia, Pa.

(2) W. H. Stein and S. Moore, *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 179 (1950).

(3) S. M. Partridge, *Biochem. J.*, **44**, 521 (1949).

(4) D. T. Englis and H. A. Fiess, *Ind. Eng. Chem.*, **36**, 604 (1944).

of equilibria corresponding to a range of values of A/B. For this purpose equation 2b is rearranged to give

$$\frac{1}{B} = \frac{1}{mT_R K}\left[\frac{T_C}{b} + (K - 1)\right] \qquad (3)$$

The curve relating $1/B$ to T_C/b will be a straight line if the process is a simple exchange. The intercept of the line, at $T_C = b$, will be $1/mT_R$ and its slope will be $1/mT_RK$.

Experimental

Materials.—The resins studied were cross-linked condensation products of formaldehyde with, (a) a polyphenol containing methylenesulfonic acid groups (Amberlite IR 100), (b) a monophenol with nuclear sulfonic acid groups (Amberlite IR 120), and, (c) a quaternary amine (Amberlite IR A-400). The IR 100 was in the form of irregular shaped particles of 10–30 mesh. The particles of IR 120 and IR A-400 were spherical and had been screened to 20–40 mesh.

The samples were cleaned by cycling with dilute sodium carbonate (IR 100 and IR 120) or dilute sodium hydroxide (IR A-400), followed with water and then with dilute hydrochloric acid. The cation exchangers were, thereby, prepared in the hydrogen form and the anion exchanger in the form of its chloride. After exhaustive washing, the samples were dried in air at room temperature and stored in tightly stoppered bottles. Moisture contents (dry weight at 110°) and exchange capacities were determined. The capacities of the cation exchangers were derived from measurements of the amounts of acid released when known weights of the resins were extracted repeatedly with dilute sodium chloride. The capacity of the anion exchanger was based on the amount of chloride removed by exhaustive extraction with sodium hydroxide.

Procedure.—The equilibria in three distinct reaction systems were studied.

Series I.—Weighed amounts of IR A-400 (0.1–0.5 g.) were equilibrated with measured volumes of 0.02 M solutions of each of seven selected amino acids. Known amounts of sodium hydroxide were added to bring the solutions to a pH close to the value of the pK_2 of the amino acid concerned. The mixtures were rocked gently until equilibrium was attained. The chloride concentration in the solution was then determined. In a number of cases the concentration of amino acid anion was also determined by titrating the solution to pH 6. Agreement between the two results confirmed the assumption that the reaction was a simple ion exchange.

Under the conditions of operation, T_C is equal to the concentration of added sodium hydroxide, and $[Cl] = a = T_C - b$. Equation 2a or 2b may, therefore, be applied to derive a constant for the exchange of the amino acid anion for the chloride ion. This constant will be designated K_{Cl}. For some purposes it may be desirable to employ the resin in the form of its hydroxide. The constant (K_{OH}) for the exchange of the amino acid anion for the hydroxyl ion may readily be obtained from the relation $K'K_{Cl} = K_{OH}$. In this relation K' is the exchange constant for the ion pair, chloride–hydroxide. A value for $1/K'$ was obtained by measurement of a series of equilibria of the resin chloride with solutions of sodium hydroxide. This gave a value of 8.1 for K'.

Series II.—Weighed amounts of IR 100 (1 g.) or of IR 120 (0.25 g.) were equilibrated with a constant volume of 0.5 or 1 M hydrochloric acid containing varying concentrations (0.02–0.2 M) of the selected amino acid. The equilibrium concentrations of amino acid in solution (T_B) were estimated colorimetrically with the aid of ninhydrin[5] or of 1,2-naphthoquinone-4-sulfonic acid.[6] The pertinent relations are $T_C = [HCl]$, $B = T_{B0} - T_B$ and $a = T_C - b$. Since the concentration of acid is high the amino acid is almost completely ionized as the cation and b is approximately equal to T_B. More precisely, $b = T_B[H^+]/[K_1 + (H^+)]$ where K_1 is the first ionization constant of the amino acid. The constant derived with the aid of equation 2b will be designated K_H since it describes the exchange of the amino acid cation for the hydrogen ion.

(5) W. Troll and R. K. Cannan, *Federation Proc.*, **10**, 260 (1951).

(6) W. Troll and R. K. Cannan, unpublished.

Series III.—Weighed amounts of IR 100 (1 g.) or of IR 120 (0.25 g.) were equilibrated with dilute unbuffered solutions (0.001–0.02 M) of each of the neutral amino acids and the equilibrium concentrations of amino acid in solution were determined as in Series II.

In Series III the amino acid is present almost entirely as the dipolar ion. The reaction with the exchanger may be formulated either as the direct addition of the dipolar ion to the resin or as an exchange with the small concentration of amino acid cation. In the latter case, equation 2a may be employed by substituting

$$b/a = b/[H^+] = T_B/K_1$$

The resulting expression can be written

$$B = \frac{mT_R K_n T_B}{1 + K_n T_B} \qquad (4)$$

where $K_nK_1 = K_H$. This equation is that of a conventional isotherm for the reaction

$$RH + H_3{}^+NCHR'COO^- = RH_3NCHR'COOH$$

and K_n is the adsorption constant for the binding of the amino acid dipolar ion by the acid form of the resin.

Concentrations rather than activities have been used in the derivation of the various constants. Since no rational evaluation of activity coefficients in the solid phase can be made, it is appropriate to ignore them in solution. Actually, we are concerned, in each phase, with the activity coefficient ratio of a pair of univalent ions. It will not be expected that this ratio will deviate much from unity in the solutions employed. In the solid phase, on the other hand, it is to be expected that differences in short range forces as one amino acid replaces another will lead to differences in ion activities. These will be reflected in the K-values that are computed on a stoichiometric basis. Indeed, the observed differences in the values of K of the individual amino acids may be interpreted as differences in the activity coefficient ratios at the surface of the exchanger of the pairs of amino acid ions that are being compared.

Results of Equilibrium Studies.—The rates of reaction varied with the nature of the resin and of the amino acid. Amberlite IRA-400 reacted most rapidly, attaining equilibrium with all amino acids in less than one hour. The equilibrium times in Series II varied from 1 to 4 hours with IR 100 and from 2 to 6 hours with IR 120, increasing with the size of the amino acid molecule. The equilibrium times in Series III were about twice as long as those in Series II.

The series of equilibria with each amino acid covered an extent of exchange varying from about 10 to 50% of the capacity of the resin. This range was adequate for satisfactory graphic analysis based on equation 3. In all cases $1/B$ proved to be a linear function of T_C/b from which K and T_R were derived as described above. The values of T_R obtained graphically from the equilibrium measurements agreed with those derived independently (Table I) in all cases except those of

TABLE I

MOISTURE, CAPACITY AND EQUIVALENT WEIGHT VALUES OF AMBERLITE RESINS

Amberlite	IR 100	IR 120	IRA-400
Moisture %, air dry resin	15.9	22.1	8.42
Capacity, mequiv./g. dry wt.	1.83	4.89	2.41
Equivalent wt.	546	204	415

TABLE II

LOGARITHMS OF EXCHANGE CONSTANTS

Resin Amberlite	Series	Glycine	Alanine	Threonine	Hydroxyproline	Proline	Leucine	Phenylalanine
				log K_H				
IR 100	II	0.38	0.43	0.27	0.52	1.07	0.97	1.70
	III	.49	.48	.34	.54	1.06	1.06	1.70
IR 120	II	.30	.30	.03	.08	0.42	0.51	1.20
	III	.32	.31	− .08	− .06	0.44	0.51	1.20
				log K_{Cl}				
IRA-400	I	−0.92	−0.88	−1.17	−1.00	−0.66	−0.58	−0.09
				log K_{OH}				
IRA-400	I	−0.01	0.03	−0.26	−0.09	0.25	0.33	0.82
				log K_n				
IR 100	III	2.83	2.82	2.55	2.46	3.05	3.42	3.53
IR 120	III	2.66	2.65	2.13	1.86	2.44	2.87	3.03
IRA-400	I	4.39	4.34	4.59	4.18	3.65	4.73	5.69
pK_1'		2.34	2.34	2.21[a]	1.92	1.99	2.36	1.83
pK_2'		9.60	9.69	9.15[a]	9.73	10.60	9.60	9.13

[a] These are the constants for serine. Those for threonine do not appear to have been measured.

leucine and phenylalanine in Series II and III. In Series II the value for IR 120 with phenylalanine was 2.57 meq. per g. dry weight. The value for IR 100 with leucine and phenylalanine was 1.43. In Series III both leucine and phenylalanine again gave a value of 1.43 for IR 100 while both gave 3.47 for IR 120. These anomalous results presumably indicate that some of the binding sites in these two resins were relatively inaccessible to the larger amino acid molecules.

The water contents, capacities and equivalent weights of the three resins are given in Table I. In Table II will be found the logarithms of the experimental values of K. The values of log K_H for Series II were obtained directly from equation 2b, those for Series III were derived from log K_n using the values of pK_1' given by Cohn and Edsall.[7] The two sets of values agree reasonably well. In Series I the values of log K_{Cl} derive directly from the equilibrium measurements, those of log K_{OH} were obtained from the relation $K'K_{Cl} = K_{OH}$ using our experimental value, log $K' = 0.91$. Computed values of K_n for IRA-400 have also been included in the table since they should reflect the behavior of the amino acids when their chromatograms on the hydroxide form of the resin are developed with buffered solutions less alkaline than pK_2. The reaction defined by K_n, in this case, is the adsorption of the amino acid dipolar ion at the cationic groups of the resin. K_n is given by $K_n = K_{OH}K_2/K_w$.

In general, the constant for a particular resin increases with increase in size of the amino acid side-chain, but is depressed by the introduction of a hydroxyl group. The three resins do not show strictly parallel specificities. The two cation exchangers have similar affinities for the smaller amino acids but IR 100 definitely binds the larger amino acids more firmly than does IR 120. As might be expected from the nature of the charged groups on the resins, IR 120 exchanges the hydrogen ion for an amino acid more readily than IRA-400 exchanges the hydroxyl ion for the same amino acid. On the other hand, when the ratios of the constants for the two resins are compared it will be found that their relative affinities for the series of amino acids are essentially parallel.

From the point of view of chromatography, it is evident that ion exchange should be very effective since a difference in log K for two components of as little as 0.1 should make separation possible. The sequence of amino acids in an elution chromatogram will depend not only on the resin employed but also on the conditions under which it is operated. The sequence of increasing values of K_H represents the expected chromatographic sequence when an amino acid mixture is eluted from a column of IR 100 or of IR 120 with dilute hydrochloric acid. When elution is carried out with buffers of such pH as to suppress the greater part of the ionization of the amino acids the chromatographic pattern may be expected to follow the sequence of values of K_n rather than of K_H. Moore and Stein[8] using a nuclear sulfonic acid resin, have employed such methods of elution and have, in fact, obtained chromatograms with an amino acid sequence corresponding to that of K_n for IR 120. To illustrate the chromatographic significance of Table II we have summarized in Table III the anticipated sequences of amino acids in columns of the three resins when K_H, K_n and K_{OH}, respectively, determine behavior. The reader will have observed (Table II) that the constants for glycine and alanine are practically the same. Yet it is known that these two amino acids can be separated on ion exchange columns. It must be concluded that the precision of equilibrium measurements is not sufficient to establish with certainty small differences in exchange affinities which may be chromatographically significant. The data for K_n with IRA-400 would indicate that alanine should precede glycine in a chromatogram when eluted with acid from an anion exchanger. Partridge and Brimley[9] have actually observed this to occur.

(7) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 84.

(8) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

(9) S. M. Partridge and R. C. Brimley, *Biochem. J.*, **49**, 153 (1951).

TABLE III

AMINO ACID SEQUENCE

Resin, Amberlite	IR 100		IR 120		IRA-400	
Constant	K_H	K_a	K_H	K_a	K_{OH}	K_a
Threonine	1	2	1	2	1	5
Glycine	2	3	3	4	3	4
Alanine	3	3	4	4	4	3
Hydroxyproline	4	1	2	1	2	2
Leucine	5	6	6	6	6	6
Proline	6	5	5	3	5	1
Phenylalanine	7	7	7	7	7	7

Chromatographic Studies

The chromatograms of proline, hydroxyproline and sarcosine to be described below were undertaken as preliminary experiments in a study of the imino acids of urine. They are included in this paper as illustrations of the relations between exchange constants and chromatographic behavior in ion exchange columns.

Equations for Retardation Volume.—The equilibrium theory of the chromatographic behavior of a single solute, whose adsorption follows a Langmuir isotherm, has been developed by De Vault,[10] Weiss[11] and Glueckauf.[12] Representing the isotherm by $B = \alpha b/(1 + \beta b)$, the retardation volume (v) in ml. is given by

$$\sqrt{v} = \sqrt{\alpha x} - \sqrt{\beta s} \qquad (5)$$

where x is the length of the column in cm., α and β are constants and s is the millimoles of solute in the column. Applying this to a uni-univalent ion exchange (equation 2b) we may equate α with MT_RK_H/T_C and β with $(K_H - 1)/T_C$, in which M represents the mass of resin in unit length of the column. Introducing these constants into equation 5 we have

$$\sqrt{v} = \frac{1}{\sqrt{T_C}}[\sqrt{MT_RK_Hx} - \sqrt{(K_H - 1)s}] \qquad (6)$$

Provided s is small compared with MT_Rx, it will be seen that the retardation volume will vary with the magnitude of the exchange constant and with the capacity of the column and will vary inversely with the concentration of acid used to develop the chromatogram. The final term can, however, be neglected only if K_H has a value very close to unity or if the capacity of the column is at least a thousand times the amount of the component.

It is inexact to apply the relation for a single solute to experiments involving more than one component. Coates and Glueckauf[13] have developed relations for multiple components. These have not been applied because their solution is tedious and our purpose was to determine the value of the simple relation for a single solute as a guide to the interpretation of chromatograms.

Experimental

The resins used were the hydrogen forms of Amberlite IR 100, Amberlite IR 120 and Dowex 50. Small columns of 5 or 25 g. of resin were prepared in buret tubes. They were washed with molar hydrochloric acid and the pore volume of each column was determined by measuring the acid recovered when the column was exhaustively washed with water. A solution of the amino acids was applied in a volume of 1 to 4 ml. and was drawn into the column. The chromatogram was then developed with 5 M (IR 100) or 2 M (IR 120 and Dowex 50) hydrochloric acid. Serial samples of the eluate of about 0.5 or 1 ml. were collected with the aid of a Technicon Fraction Collector. The exact volumes were computed from the drop count and a calibration of the drop volume in each experiment. After neutralization of each fraction, the amino acid concentration was determined colorimetrically using 1,2-naphthoquinone-4-sulfonic acid.[6]

Results of Chromatographic Studies.—The characteristics of the three columns and the operating conditions are detailed in Table IV. The elution curves are shown in Figs. 1 and 2, where the amino acid concentration is plotted as a function of the volume of eluate after subtraction of the pore volume.

TABLE IV

CHROMATOGRAPHIC COLUMNS

Resin	Amberlite IR 100	Amberlite IR 120	Dowex 50
Weight of resing, g.	5	5	25
Dimensions of column, cm.	16 × 1.1	11.2 × 1.1	36 × 1.2
Pore volume, ml.	10.0	3.7	14.0
Capacity, meq.	9.0	19.1	91.7
Flow rate, ml./hr./cm.^{2}	10.5	2.7	1.9
Eluting solution, HCl, M	5	2	2
Particle size, sieve mesh	100	325	325

Five runs were made as indicated in Table V, which records, also, the observed retardation volumes together with those calculated from the exchange constants of the amino acids using equation 6.

TABLE V

RETARDATION VOLUMES

Run	Resin	Amino acids, millimoles	Retardation volume, Obsd.	Retardation volume, Calcd.	Recovery, %
1	IR 100	0.03 proline	31.0	18.8	83.8
		0.03 hydroxyproline	8.5	5.4	105.8
2	Dowex 50	0.05 proline	130	117	100.2
		0.033 sarcosine	76	...	98.8
		0.0167 hydroxyproline	59	55	98.8
3	IR 120	0.03 proline	26.3	23.6	103.5
		0.03 hydroxpyroline	11.3	11.1	97.6
4	IR 120	0.30 proline	19.8	20.4	104.4
		0.007 hydroxyproline[a]	10.9	11.3	
5	IR 120	1.5 proline	14.0	15.3	100.4
		0.033 hydroxyproline[a]	9.5	11.1	

[a] Impurity in the proline.

The calculated volumes for Dowex 50 are based on the assumption that the constants for this resin are the same as those for IR 120. In the fourth and fifth run proline alone was applied to the column. The chromatograms, however, betrayed the presence of 2.4% of hydroxyproline as an impurity. Calculated retardation volumes for this unexpected component are included in Table V.

It will be seen that the Dowex 50 column gave the most satisfactory results although the much smaller column of Amberlite IR 120 at a comparable flow rate was also satisfactory. The column of Amberlite IR 100 gave broad diffuse bands with evidence of extensive tailing. These effects are evidence of lack of equilibrium resulting from the

(10) D. De Vault, THIS JOURNAL, **65**, 532 (1943).
(11) J. Weiss, *J. Chem. Soc.*, 297 (1943).
(12) E. Glueckauf, *ibid.*, 1302 (1947).
(13) J. I. Coates and E. Glueckauf, *ibid.*, 1308 (1947).

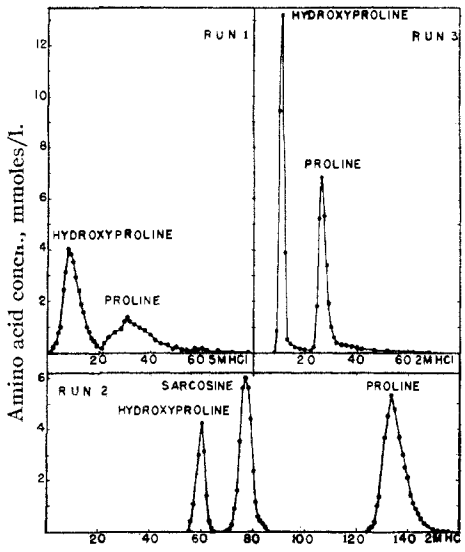

Fig. 1.—Effect of flow rate, resin particle size, and amount of resin on the separation of amino acids, using columns of Amberlite IR 100 (run 1), Dowex 50 (run 2), and Amberlite IR 120 (run 3).

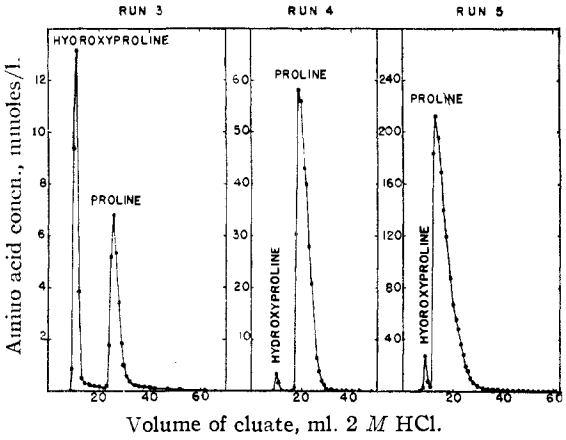

Fig. 2.—Effect of load on retardation volumes of amino acids, eluted from columns of Amberlite IR 120, using a mixture of 0.03 millimole of proline and 0.03 millimole of hydroxyproline (run 3), 0.3 millimole of proline (run 4), and 1.5 millimoles of proline (run 5).

combined influences of high flow rate and relatively large particles. Some channeling, due to uneven packing of the irregularly shaped particles may, also, have contributed to the poor results in the case of Amberlite IR 100.

The degree of agreement between the observed and the calculated retardation volumes for the Amberlite IR 120 and the Dowex 50 columns may be regarded as satisfactory in view of the approximations involved and the lack of precision in the values of K_H. In the case of Dowex 50, this agreement confirms the opinion that the exchange characteristics of this resin are similar to those of Amberlite IR 120.

In precision chromatography it is desirable to work with a large value of MT_Rx/s. It is evident, however, from the fifth run that good separations can be achieved at remarkably low values of this ratio provided the exchange constants of the components are well separated. In this run 0.173 g. of proline was separated from the hydroxyproline on a column of 5 g. of resin. That is to say a column with a capacity of 19 millimoles was adequate to resolve 1.5 millimoles of the amino acid. Elution chromatography on ion exchange columns promises to be valuable in preparative work as well as in the analysis of amino acid mixtures.

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A Procedure for the Ion Exchange Chromatography of the Amino Acids of Urine[1]

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A method is presented for the chromatographic separation of the amino acids in urine. Ion exchange procedures are used for the removal of salts in a first step and the removal of most of the nitrogenous substances in a second step. Thereby, a crude ampholyte fraction is obtained without significant loss of amino acids. Separation of the single amino acids in this fraction is effected in a third step by ion exchange chromatography. The single amino acids can be identified by paper chromatography and by specific tests.

The overwhelming amounts of inorganic ions and of nitrogenous bases in biological fluids such as urine have been found to interfere with the chromatography of the amino acids on paper and on starch columns. Anticipating similar problems in the use of ion exchange columns we explored a method of preliminary desalting by ion exchange followed by precise chromatography on a cation exchange column. Recently, a similar procedure for removing salts has been applied by Piez, Tooper and Fosdick prior to paper chromatography.[2] On the other hand, Moore and Stein[3] avoided the desalting by using a sulfonated cation exchanger in its sodium form to chromatograph urinary amino acids. In their procedure, elution of the amino acids is effected with a series of buffers of increasing pH and resolution is improved by periodic modification of the temperature of the column. Our preliminary desalting has made it possible to eliminate the exacting control of pH and temperature neces-

(1) A preliminary report was presented at the Meeting of the American Society of Biological Chemists: M. E. Carsten, *Federation Proc.*, **10**, 170 (1951).

(2) K. A. Piez, E. B. Tooper and L. S. Fosdick, *J. Biol. Chem.*, **194**, 669 (1952).

(3) S. Moore and W. H. Stein, *ibid.*, **192**, 663 (1951).