a, R = COOC$_2$H$_5$
b, R = Cl
c, R = H

The action of alkali or ammonia on Ib·HCl (m.p. 210–215°; $\lambda_{max}^{H_2O}$ 241 m$\mu$, log $\epsilon$ 4.16; *Anal.* Calcd. for C$_{11}$H$_{14}$N$_5$Cl·HCl: C, 45.84; H, 5.25; N, 24.31. Found: C, 45.98; H, 5.17; N, 24.48) affords IIb[2,3] (m.p. 130–133°; $\lambda_{max}^{H_2O}$ 255 m$\mu$, log $\epsilon$ 4.25), 6-amino-2 - (*p* - chloroanilino) - 1,4 - dihydro - 4,4 - dimethyl-*s*-triazine,[4] by an irreversible intramolecular rearrangement. IIb·HCl (m.p. 128–131°; $\lambda_{max}^{H_2O}$ 255 m$\mu$, log $\epsilon$ 4.24; *Anal.* Calcd. for C$_{11}$H$_{14}$N$_5$Cl· HCl: C, 45.84; H, 5.25; N, 24.31; Cl, 24.61. Found: C, 45.67; H, 5.21; N, 24.53; Cl, 24.70), isomeric with Ib·HCl, results on treatment of IIb with hydrochloric acid.

Many compounds of structure I inhibit *Streptococcus faecalis 8043* and *Lactobacillus arabinosus 17-5* in standard pteroylglutamic acid and niacin assay systems,[5] whereas those of structure II are relatively inert. For example, Ib·HCl (0.05–0.20 gamma/ml.) inhibits 0.001–0.01 gamma/ml. of pteroylglutamic acid or niacin in corresponding microbiological systems, whereas IIb produces only partial inhibition versus 0.001 gamma/ml. of pteroylglutamic acid or niacin in concentrations of 100–1000 gamma/ml. Ib·HCl also inhibits *Lactobacillus casei 7469* and *Leuconostoc citrovorum 8081* in systems containing 0.001–0.01 gamma/ml. of riboflavin[5] and 0.6 unit/ml. of citrovorum factor[6] respectively, but only in concentrations of 100 gamma/ml. or more. Other derivatives of structure I exhibit similar activities, although the minimal inhibiting dose varies with substitution in the molecule while, in general, derivatives of structure II are inert.

Inhibition induced in pteroylglutamic acid systems by appropriate concentrations of compounds of structure I differs from that obtained with 4-amino-pteroylglutamic acid (0.1 gamma/ml. or more) in that it is irreversible by 0.1–200 gamma/ml. of pteroylglutamic acid, adenine, or guanine. Reversal is obtained with citrovorum factor (0.1 unit/ml.), leucovorin (1.0 unit/ml.), dihydro-pteroylglutamic acid (0.01 gamma/ml.), N$^{10}$-formyl-pteroylglutamic acid (0.01 gamma/ml.), thymine (10 gamma/ml.), certain components of nucleic acid or high concentrations of ascorbic acid (200 gamma/ml.). When substituted for pteroylglutamic acid in *Streptococcus faecalis 8043* systems, citrovorum factor (0.000001 unit/ml.), leuco-

(2) S. Birtwell, F. H. S. Curd, J. A. Hendry and F. L. Rose, *J. Chem. Soc.*, 1645 (1948).

(3) N. N. Crounse, *J. Org. Chem.*, 16, 492 (1951).

(4) Or a tautomer of this structure.

(5) Assoc. Vitamin Chemists, Inc., "Methods of Vitamin Assay," 2nd ed., Interscience Publishers, New York, N. Y., 1951.

(6) H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, 176, 165 (1948).

vorin (0.001 unit/ml.), dihydropteroylglutamic acid (0.001 gamma/ml.) and N$^{10}$-formylpteroylglutamic acid (0.00001 gamma/ml.) are inhibited only by those concentrations required to inhibit *Leuconostoc citrovorum 8081* (*circa* 100 gamma/ml.). No significant inhibition was observed versus thymine.

Preliminary tests indicate that several compounds of structure I exhibit activity against experimental avian malaria, although compounds of structure II are comparatively inactive. For example, Ib·HCl is active against *Plasmodium lophurae* in the duck at oral dosages of approximately 3.13 mg./kg. and Ic·HCl at approximately 12.5 mg./kg. Ib·HCl is about six times as active as quinine, twice as active as atabrine or paludrine, and half as active as plasmochin in the duck.[7]

Further extensive studies on the scope and mechanism of the reaction and on the biological activity and mode of action of these compounds are in progress.

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## QUANTITATIVE CALCULATION OF THE BEHAVIOR OF ELUTION BANDS IN THE SEPARATION OF RARE EARTHS ON ION-EXCHANGE COLUMNS

*Sir:*

This Laboratory, in a series of papers, has described procedures for separating rare earths on ion-exchange columns. In the accompanying letter a quantitative theory for describing this phenomenon in the *p*H range between 5.5 and 7.3 for 0.1% citric acid has been outlined. In the course of this work, some very interesting quantitative relationships concerning the movement of bands on the column have been observed. Since the high-capacity resins used have a constant equivalent capacity for the ions adsorbed, if the number of equivalents of rare earth on the column is known, then the length of the original rare earth band can be calculated. If this band is eluted down the column with a citrate solution of known *p*H, the band widens out until it reaches an equilibrium value and from then on the front edge of the band travels at the same rate as the rear edge. Within this band the rare earths separate into individual bands proportionate to the amount of rare earth present and in an order starting with the heavier rare earths and proceeding to the lighter rare earths. Under equilibrium conditions these bands are exceedingly sharp. However, in practice they tend to be blurred due to channeling and non-equilibrium conditions. Under very carefully controlled procedures they can be made very sharp. We expect to publish shortly detailed experimental results and calculations showing that it is possible to calculate to better than 5%, and in most cases to better than 1 to 2%, the length of the equilibrium band on the column, the rate at which the front and rear edges

of the band progress down the column, the length of the column necessary to reach the fully developed band and the number of liters of citric acid which will have to be passed through the column before the break-through of the rare earths at the bottom of the column is observed.

Experimental results will be presented correlating the shrinkage or expansion of the resins with the form of the resin present: for example, rare earth resin, ammonium resin and hydrogen resin. It has been possible to determine these band fronts much more precisely when neodymium was used since band edges become very clearly visible, due to the characteristic color of the neodymium ions, when the columns are illuminated in the dark with blue light.

Since the over-all change which occurs when a rare earth equilibrium band is being moved down the column is the conversion of the hydrogen resin which precedes the band into the ammonium resin which follows it, the break-through of the rare earth band at the bottom can be calculated with very high precision. While there is of course some hydrogen resin mixed with the ammonium resin which follows the band, the amount of this resin is extremely small as can be readily seen from the fact that the hydrogen ion in solution is so dilute compared to the ammonium ion. Accordingly, one needs only to divide the number of active points in the column occupied by hydrogen below the originally adsorbed rare earth band by the number of ammonium ions which are being put in the top of the column per liter to obtain the break-through volume. For example

$$V_B = \frac{A - B}{C_{NH_4^+}} = \frac{WQ - B}{C_{NH_4^+}}$$

$V_B$ = volume of eluant required for break-through in liters
$A$ = exchange capacity of the resin bed in milliequivalents
$B$ = milliequivalents of rare earth adsorbed on bed
$C_{NH_4^+}$ = milliequivalents of ammonium ion per liter of eluant
$W$ = weight of resin in the column in grams
$Q$ = capacity of the resin in milliequivalents per gram

| pH | Rare earth | A meq. | B meq. | A − B meq. | $C_{NH_4^+}$ meq./l. | $V_B$ (calcd.), l. | $V_B$ (obs.), l. |
|---|---|---|---|---|---|---|---|
| 6.4 | Pr | 852 | 44.0 | 808 | 13.87 | 58.2 | 58.0 |
|  | Nd | 852 | 44.5 | 807 | 13.87 | 58.1 | 58.1 |
| 6.1 | Pr | 852 | 44.0 | 808 | 12.90 | 62.6 | 62.5 |
|  | Nd | 852 | 44.5 | 807 | 12.90 | 62.5 | 62.4 |
| 5.8 | Pr | 852 | 44.0 | 808 | 11.78 | 68.6 | 68.5 |
|  | Nd | 852 | 44.5 | 807 | 11.78 | 68.5 | 68.4 |
| 5.5 | Pr | 852 | 44.0 | 808 | 10.69 | 75.6 | 75.4 |
|  | Nd | 852 | 44.5 | 807 | 10.69 | 75.5 | 75.4 |
| 7.3 | Nd | 852 | 178.0 | 674 | 15.38 | 43.8 | 43.7 |
| 7.6 | Nd | 852 | 178.0 | 674 | 15.70 | 42.9 | 43.0 |
| 8.2 | Nd | 852 | 178.0 | 674 | 16.88 | 39.9 | 39.8 |
| 5.6 | Nd | 852 | 27.0 | 825 | 10.99 | 75.1 | 75.0 |
| 5.9 | Nd | 852 | 53.5 | 798 | 12.10 | 65.9 | 65.5[a] |
| 6.2 | Nd | 852 | 89.0 | 763 | 13.27 | 57.5 | 57.5 |
| 6.5 | Nd | 852 | 89.0 | 763 | 14.05 | 54.3 | 54.0 |
| 6.8 | Nd | 852 | 89.0 | 763 | 14.67 | 52.0 | 51.8[a] |

[a] Extrapolated values obtained from band-front measurements.

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## QUANTITATIVE THEORY OF RARE EARTH SEPARATIONS ON ION-EXCHANGE COLUMNS

*Sir:*

This Laboratory, in a series of papers (THIS JOURNAL, **69**, 2812 (1947); **72**, 2349, 2354 (1950); **73**, 4840 (1951)) has described how the rare earths can be separated from each other on ion-exchange columns. The method consists essentially of adsorbing a mixture of neutral rare earth chlorides on the top of a high-capacity ion-exchange column in the hydrogen cycle. The rare earth band which forms at the top is then moved down the column by eluting it with a solution of citric acid which has been adjusted to a known $pH$ value by the addition of ammonium hydroxide. If the $pH$ range of the eluant is between 5.5 and 7.3 for 0.1% citric acid, it is found that when the elution curves are plotted (concentration of rare earth in the eluate against liters of citric acid passed through the column), flat-topped elution curves are observed.

Shortly we expect to publish detailed experimental results and calculations showing that if the amount of rare earth adsorbed on the column, the concentration of citrate solution which goes in the top of the column and the $pH$ of that solution, or its ammonium equivalent, are known, then it is possible to calculate to better than 5%, and in most cases to better than 1 or 2%, the composition of the eluate coming out the bottom. This includes the ammonium concentration, the rare earth concentration and the $pH$ or hydrogen ion concentration. Some very interesting relations between the data and the activity coefficients of the resin can be observed. These can be deduced as a result of the constraints put upon the system by the fact that the resin has a fixed capacity. In order to make these calculations it is only necessary to use simple thermodynamics involving the ionization constants $K_1$, $K_2$, $K_3$ of citric acid, the equilibrium constant of the rare earth complex, the conservation conditions of the electrical neutrality of the solution and the fixed capacity of the resin.

It has been found that the predominating and only important rare earth complex formed in this $pH$ range is (RE cit$_2^\equiv$). Experimentally, it has been found that if the ammonium ion of the eluant, the hydrogen total of the eluant $H_T$ (a summation of the hydrogen combined with $H_3$cit, $H_2$cit$^-$ and Hcit$^=$ + the $H^+$ ion), the $H_T$ of the eluate, the ammonium ion of the eluate, and the rare earth total of the eluate are plotted against the $H^+$ ion of the eluate, that linear curves result. Accordingly, if any pair of these are plotted against each other linear functions are also found. The slopes of these lines can be calculated from theoretical considerations. Furthermore, the instability constant $K$ for the RE cit$_2^\equiv$ complex can be calculated for each of the rare earths.

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