metal was lost to the walls of the distillation tube, and some remained in the samarium lean fractions which were not redistilled.

About 30 g. of high purity samarium metal has been prepared by the method outlined here.

Undoubtedly calcium could be used for partial separation of the rare earths by fractional distillation of the metals from a calcium–rare earth oxide mixture. Spedding and Daane[5] have shown that lanthanum is quite effective for this type of separation.

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[5] F. H. Spedding and A. H. Daane, "Methods of Producing Rare Earth Metals as Developed at Iowa State College," Paper presented at the Annual Meeting of the AIME (Feb. 1954).

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Anion-exchange Studies. XIV. The Alkali Metals in Ethylenediaminetetraacetic Acid Solutions[1,2]

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The alkali metals, lithium and sodium, have been shown to form reasonably stable complexes with the chelating agent, ethylenediaminetetraacetic acid, at high pH[3] according to the equilibrium

$$M^+ + A^{-4} \rightleftarrows MA^{-3} \qquad (1)$$

where M^+ is the alkali ion and A^{-4} is ethylenediaminetetraacetate (EDTA). The concentration quotients $k^m_{01} = m_{MX^{-3}}/m_{M^+}m_{A^{-4}}$ were estimated to be $k^m_{01} = 620$ for Li^+ and $k^m_{01} = 46$ for Na^+ at 20° in 0.1 *M* KCl. Under the same conditions negligible complexing of potassium ions was assumed. One might also expect negligible complexing of cesium and rubidium ions by EDTA. The apparent formation of negatively charged complexes of lithium and sodium in alkaline EDTA solutions suggested that their separation from each other and from other alkali metals might be accomplished by anion exchange in a manner similar to the separation of the alkaline earths in citrate solutions which was described earlier.[2] In this connection it might be mentioned that the effect of another chelating agent, uramil-diacetic acid[4] in the cation-exchange separation of alkali metals was recently investigated.[5] Although uramil-diacetate forms better complexes with lithium and sodium than EDTA (compare references 3 and 4), the latter was chosen for the anion exchange experiments because of its easier availability.

The experiments were carried out with a strong base quaternary amine polystyrene divinylbenzene resin (Dowex-1) of relatively low cross-linking (4%) and mesh size 50–100. EDTA solutions were prepared by dissolving ethylenediaminetetraacetic acid (H_4A) in KOH solutions. The resin, initially in the chloride form, was treated with *ca.* 0.25 *M* EDTA solution of pH *ca.* 11. At this pH, H_4A may be considered to be largely dissociated to A^{-4}[6] and thus the resin treated with 0.25 *M* EDTA of pH 11 might be expected to be principally in an $A^{-4} - HA^{-3}$ form with A^{-4} possibly predominating. After the resin had been treated with several volumes of alkaline EDTA solution, it was washed with water to remove excess EDTA. A portion of the resin was then analyzed for chloride. It was found that the resin retained significant amounts of chloride which could be removed only slowly by further washing with EDTA solution.

In a typical experiment designed to demonstrate separation of alkali metals, an 0.41 cm.² × 23 cm. column of this resin was pretreated with *ca.* 2 column volumes of 2.5×10^{-3} *M* EDTA solution at pH 10.9. A 1.0-ml. aliquot of a solution containing 0.12 *M* LiOH, 0.015 *M* NaOH (Na^{22}), Cs^{137} and 0.13 *M* EDTA at pH 10 was then added to the column and elution was carried out with the same dilute EDTA solution which was used to pretreat the column. The effluent was analyzed radiometrically for Cs^{137} and Na^{22} and spectrographically or flame spectrophotometrically for Li.[7]

As shown in Fig. 1, cesium appeared in the effluent first, essentially unadsorbed, and was satisfactorily separated from sodium which was slightly retained by the column. It is predicted that rubidium and potassium ions in this medium would also have shown negligible adsorption. The cesium and sodium fractions were analyzed spectrographically and found to be free of lithium within the limits of the spectrographic method. Satisfactory elution of lithium was achieved with 0.25 *M* EDTA solution at pH 4.2. Effluent solutions were analyzed by flame spectrophotometry.[7] Appearance of lithium in the effluent was paralleled by a decrease in pH as shown by the dashed line in Fig. 1. This decrease in pH was delayed as shown in Fig. 1, suggesting that acid ions of EDTA (*e.g.*, $H_2A^=$) are adsorbed by the resin as expected on the basis of earlier work on acid adsorption by "strong base" resins containing polyvalent anions.[8]

Adsorption of base can also be demonstrated with this "strong base" resin in the EDTA form. Thus when the resin was pretreated with a 0.25 *M* EDTA solution near pH 11, reasonably good (total) adsorption of LiOH occurred, *e.g.*, *ca.* 3–4 column volumes of a 0.08 *M* LiOH solution could be passed into the columns before breakthrough of lithium occurred. When the resin was pretreated with *ca.* 0.25 *M* EDTA solution near pH 4.2 adsorption of lithium hydroxide also occurred, although some lithium appeared in the effluent almost immediately. These experiments suggest that adsorption of base may occur according to reactions of the type

$$(A^{-4})_r + Li^+ + OH^- \longrightarrow (Li\,A^{-3})_r + (OH^-)_r \qquad (2)$$

or

$$(HA^{-3})_r + Li^+ + OH^- \longrightarrow (Li\,A^{-3})_r + H_2O \qquad (3)$$

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[1] This document is based on work performed for the U. S. Atomic Energy Commission at the Oak Ridge National Laboratory.

[2] Previous paper: F. Nelson and K. A. Kraus, This Journal, **76**, 801 (1954).

[3] G. Schwartzenbach and H. Ackermann, *Helv. Chim. Acta*, **30**, 1798 (1947).

[4] G. Schwartzenbach, E. Kampitsch and R. Steiner, *ibid.*, **29**, 364 (1946).

[5] W. Buser, *ibid.*, **34**, 1635 (1951).

[6] *pK* values for the ionization constants of H_4A in 0.1 *N* KCl (20°) are reported as $pK_1 = 1.996$, $pK_2 = 2.672$, $pK_3 = 6.161$ and $pK_4 = 10.262$ (ref. 3). See also F. F. Carini and A. E. Martell, This Journal, **74**, 5745 (1952).

[7] We are indebted to Mr. M. Murray of the ORNL Analytical Division for the spectrographic analyses and to Mr. W. R. Laing of the same Division for the flame spectrophotometric analyses.

[8] (a) K. A. Kraus, F. Nelson and J. F. Baxter, This Journal, **75**, 2768 (1953); (b) F. Nelson and K. A. Kraus, *ibid.*, **76**, 329 (1954).

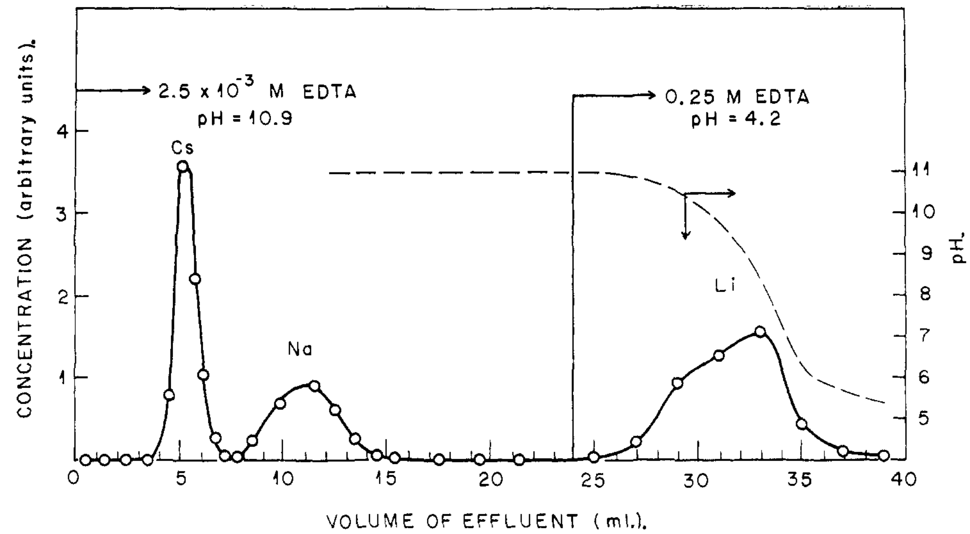

Fig. 1.—Separation of alkali metals by anion exchange.

which are analogous to the acid uptake described earlier.[8]

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COMMUNICATIONS TO THE EDITOR

THE ACTIVATION OF CHYMOTRYPSINOGEN

Sir:

It was previously reported[1] that the slow activation[2] of chymotrypsinogen is accompanied by the formation of several peptides. probably arising from a common precursor, containing 8 or 9 different amino acid residues. We now wish to report the isolation and structure of a dipeptide which is probably the only peptide liberated in stoichiometrically significant amounts during the activation of chymotrypsinogen.

Electrophoretic analysis,[3,4] end group analysis,[4] specificity requirements of the activating enzymes and other considerations[1] suggested that a basic peptide was liberated during the conversion of π-chymotrypsin to the δ-form. Hence activation mixtures were prepared under conditions yielding preponderantly δ-chymotrypsin.[4,5] The products of activation were characterized by activity measurements (esterase[6]) and by electrophoresis in acetate buffer, *p*H 4.97, usually in the presence of a 10–30 fold molar excess with respect to active chymotrypsin of diisopropylphosphofluoridate (DFP). Free peptides were isolated by applying to an ion exchange column (0.9 × 15 cm.) of XE-64, 150–400 mesh, Na$^+$ form[7] either the activation mixture in the presence of DFP, or the fraction soluble in 10% trichloroacetic acid (TCA). Elution with 0.3*M* sodium citrate buffer, *p*H 5.3, yielded a sharp peptide peak which emerged with the same effluent volume as arginine. The yield of peptide was approximately 0.8 mole per mole of chymotrypsinogen activated (molecular weight 23,000). Acid hydrolysis of the peptide yielded approximately equal amounts of serine and arginine. Analysis of the acid hydrolyzate of the entire TCA-soluble fraction yielded the same two amino acids in stoichiometrically significant amounts, in addition to much smaller amounts of numerous amino acids.[1] When the peptide was converted to the dinitrophenyl derivative[8] and subsequently hydrolyzed, only DNP-serine was obtained[9] on paper chromatograms, indicating that the peptide had the structure seryl-arginine. The same peptide was observed by paper chromatography as product of the slow activation of chymotrypsinogen, whereas it was absent under conditions yielding predominantly π-chymotrypsin, thus indicating that it could only have arisen during the π-δ

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(2) M. Kunitz and J. H. Northrop, *J. Gen. Physiol.*, **18**, 433 (1935).

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(6) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 493 (1949).

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