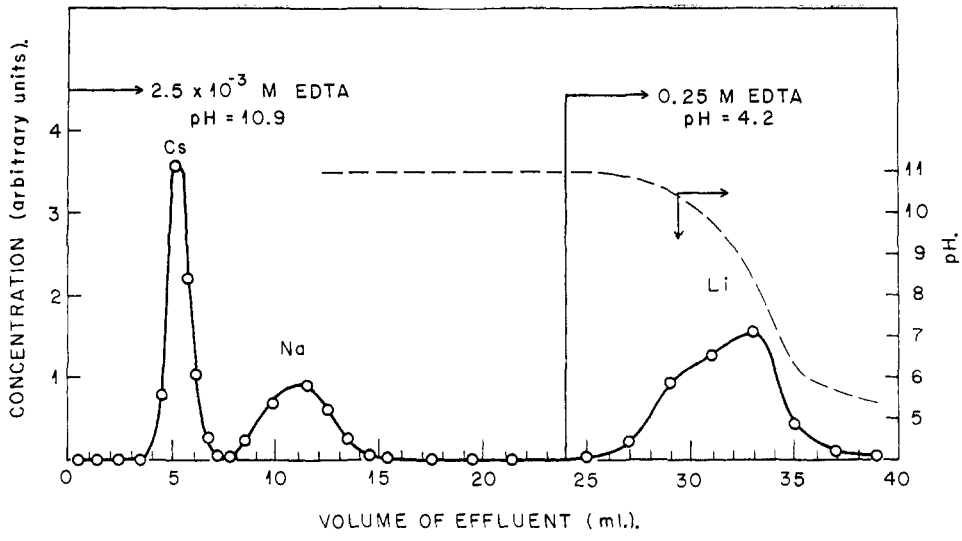

Fig. 1.—Separation of alkali metals by anion exchange.

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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conversion but that its liberation is not a prerequisite for chymotryptic activity.

On the basis of these data, and others previously reported, it now appears possible to identify the amino acid sequence in chymotrypsinogen which is primarily involved in the activation process. The following considerations summarize the pertinent points of evidence: (1) π-chymotrypsin differs from chymotrypsinogen in possessing an N-terminal isoleucyl-valine sequence,[4,10] both proteins being devoid of a C-terminal group reactive toward carboxypeptidase[4]; (2) the conversion of π- to δ-chymotrypsin yields a C-terminal leucine group, no new N-terminal group, and the dipeptide seryl-arginine; (3) the action of α-chymotrypsin on chymotrypsinogen yields neither enzymatic activity nor the dipeptide,[11] suggesting that seryl-arginine is not a C-terminal sequence in chymotrypsinogen. Omitting from consideration factors arising from the presence of the N-terminal half-cystine group found in chymotrypsinogen[12] it seems most likely that the amino acid sequence involved in activation is leucyl-seryl-arginyl-isoleucyl-valine, and that the arginyl-isoleucine bond is opened in the trypsin-catalyzed formation of π-chymotrypsin. The subsequent, chymotrypsin-catalyzed[4] conversion of π- to δ-chymotrypsin involves the hydrolysis of the leucyl-serine bond, giving rise to the dipeptide, a C-terminal leucine group and an N-terminal isoleucyl-valine sequence. It is worthy of note that, as in the activation of trypsinogen,[13] the splitting of a single bond suffices to produce enzymatic activity and that all of these active enzymes have the same N-terminal dipeptide sequence.[14] The splitting of the leucyl-serine bond of π-chymotrypsin is without effect on the specific esterase activity of the activation mixture. Details of this work will be published elsewhere.

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ISOLATION OF A DPN ISOMER CONTAINING NICOTINAMIDE RIBOSIDE IN THE α LINKAGE[1]

Sir:

It has been reported previously that treatment of diphosphopyridine nucleotide (DPN) with the DPNase from *Neurospora crassa* results in the cleavage of the nicotinamide riboside bond.[2] Although the DPNase completely destroyed all activity of the DPN for yeast alcohol dehydrogenase, there was still some remaining reaction with cyanide.[3]

It was suggested that this cyanide reacting residue might be due to the presence of nicotinamide mononucleotide or riboside. We have recently reinvestigated this residue, and have found that DPN with high purity prepared from either liver or yeast still contains material which reacts with cyanide and is resistant to the action of the *Neurospora* enzyme. However, the residue is not nicotinamide mononucleotide or riboside since the compound does not promote the growth of *Hemophilus parainfluenzae*, which will grow on either the riboside or nucleotide as well as on DPN.[4] We have now been able to isolate a compound from a number of highly purified commercial DPN preparations, which give the same analysis for adenine, nicotinamide, ribose and phosphate as DPN, but has no alcohol dehydrogenase activity. The compound was obtained by first treating the DPN preparation with the *Neurospora* DPNase, and then separating the residual cyanide reacting material from the adenosine-diphosphate ribose by column chromatography. The compound was precipitated from acid acetone. We have tentatively named this compound "DPN isomer" because of its identical analysis with DPN. From 5 g. of Pabst DPN[5] 300 mg. of the "isomer" was isolated. We have estimated the concentration of the "isomer" to be from 10 to 15% in the purified DPN.

The isomer moves at the same R_F as DPN on paper in a large number of solvents. Likewise it cannot be separated from DPN by column chromatography. The isomer can be distinguished from DPN not only by its inactivity in the alcohol dehydrogenase (yeast or liver), the muscle triosephosphate dehydrogenase and muscle lactic dehydrogenase systems, but also by its inability to serve as substrate for the *Neurospora* DPNase and as a growth factor for *Hemophilus parainfluenzae*. The compound reacts with hydrosulfite to give a product of reduction showing an absorption peak at 348 mμ; furthermore, the cyanide addition product of the isomer has a maximum absorption at about 332 mμ as compared to the 325 maximum for DPN.

DPN can serve as the nicotinamide source for a nicotinamide requiring *Neurospora* mutant. However, the isomer does not promote the growth of the mutant (Table I). On heating the isomer for 30′ at 100° (*p*H 5), the nicotinamide ribosidic bond is cleaved; and this results in growth activity for the mutant. This and other evidence indicate that the pyridine component of the isomer is nicotinamide.

Treatment of the isomer with the snake venom pyrophosphatase results in the liberation of all the adenine as 5′-adenylic acid. The nicotinamide mononucleotide moiety of the isomer has a similar cyanide addition product as the parent compound

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(5) We wish to thank the Pabst Laboratories for generously supplying some of the DPN used in these experiments.