TABLE I

HALF-WAVE POTENTIALS OF DEHYDROASCORBIC ACID IN McELVAINE BUFFER SOLUTION AT 25°

Concentration of dehydroascorbic acid, 0.025 *M*

*p*H	2.2	2.66	2.96	3.48
$\pi^1/_2$ (*vs.* N. C. E.)	−0.350	−0.372	−0.392	−0.410
*p*H	3.80	4.22	4.63	5.04
$\pi^1/_2$ (*vs.* N. C. E.)	−0.432	−0.450	−0.462	−0.480

The significance of these half wave potentials will be discussed later.

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STUDIES ON PITUITARY ADRENOCORTICOTROPIN. VI. AN N-TERMINAL SEQUENCE OF CORTICOTROPIN-A

Sirs:

We have investigated the N-terminus of two highly purified ACTH preparations by the use of the DNFB method of Sanger and also by a recently developed modification of the thiohydantoin method of Edman.[1] As used by us, the latter procedure involves the direct identification of the hydantoin by paper chromatography.[2] In applying this technique to the characterization of the fractions arising from the chromatography of unhydrolyzed hog pituitary extracts on XE-97 resin,[3] it was found that the slow-moving active peak, designated Type ID, showed a single thiohydantoin corresponding to the amino acid, serine. (By contrast, the inactive material passing directly through the column, designated Type IA, gave several different thiohydantoins.) The stepwise degradation of Type ID material was continued by a second application of the Edman reaction and again a single thiohydantoin was detected, this time corresponding to the amino acid, tyrosine. Further application of the step-wise degradation technique gave equivocal results at the third position and therefore was discontinued.[4]

When the apparently pure unhydrolyzed ACTH, designated Corticotropin-A,[5] became available the stepwise degradation technique was again applied. Again the sequence Ser.Tyr. was obtained. In order to confirm the presence of serine at the N-terminus, Corticotropin-A was treated with dinitrofluorobenzene by the method of Sanger.[6] After acid hydrolysis of the DNP-Corticotropin-A, DNP-serine was identified in the ether extract by paper chromatography. At the same time, all of the serine was absent from the amino acid spectrum of the aqueous phase.[7]

Additional evidence for the presence in Corticotropin-A of the sequence, Ser.Tyr., has been obtained by the isolation of the dipeptide from the products of the chymotryptic digestion of Corticotropin-A. This peptide, which is a major constituent of the mixture, has an R_f value (Whatman #1) of 0.43 in the Partridge system[8] and travels at a rate intermediate between tyrosine and serine in an *s*-butyl alcohol/3% ammonia system.[9] Complete acid hydrolysis gave only serine and tyrosine and digestion for 24 hours with 5% carboxypeptidase resulted in complete hydrolysis to serine and tyrosine. In order to confirm the sequence of the amino acids in the dipeptide, it was treated with DNFB and hydrolyzed. By paper chromatography of the ether extract in two systems, one developed by us,[10] and the other the *t*-amyl alcohol solvent of Blackburn and Lowther,[11] serine was identified as the terminal residue. Chromatography of the aqueous layer in *t*-amyl alcohol showed no colored DNP-amino acids. Upon treatment of the paper with ninhydrin, the characteristic greyish-blue color of O-DNP-tyrosine was readily discernible, at an R_f corresponding to that of the reference compound run on the same sheet.

Thus it appears by a combination of chemical and enzymatic evidence that an N-terminal sequence of Corticotropin-A is Ser.Tyr. Cleavage of the peptide chain to form the dipeptide Ser.Tyr. is consistent with classical concepts of the specificity of chymotrypsin.[12]

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. A. Gross.

(1) P. Edman, *Acta Chem. Scand.*, **4**, 277 (1950).

(2) W. A. Landmann, M. P. Drake and J. Dillaha, THIS JOURNAL, **75**, 3638 (1953).

(3) W. F. White and W. L. Fierce, THIS JOURNAL, **75**, 245 (1953).

(4) During the course of our work, a portion of the same preparation was given to Dr. Sidney W. Fox of Iowa State College for sequence studies by his technique (S. W. Fox, T. L. Hurst, and K. F. Itschner, THIS JOURNAL, **73**, 3573 (1951)). His results are in agreement with ours.

(5) W. F. White, THIS JOURNAL, **75**, 503 (1953). In this publication one residue of tyrosine was inadvertently omitted from the empirical formula in the fifth paragraph.

(6) F. Sanger, *Biochem. J.*, **53**, 355 (1953).

(7) The amino acids were separated by a paper chromatographic technique (J. F. Roland and A. Gross, to be published) and were developed with ninhydrin. Thus, in addition to serine, tyrosine and lysine were also missing from their usual positions due to reactions with DNFB. However, since the α-N DNP derivatives of tyrosine and lysine were not found these two amino acids were not located at the N-terminus.

(8) *n*-Butyl alcohol:acetic acid:water (80:20:100).

(9) This system is used in an extended run of 48–60 hours with an absorbent pad attached to the bottom of the sheet. Under these conditions phenylalanine, the fastest moving amino acid, has almost reached the end of a 22-inch strip. By comparison with phenylalanine Ser.Tyr. has a rate of about 0.4.

(10) Xylene/gl. acetic acid/*p*H 6.0 phthalate buffer (0.05 *M*) in volume ratios of 10:5:4. The paper, buffered with the same buffer, was equilibrated with the lower layer for sixteen hours before development with the upper layer. This system is capable of separating the DNP derivatives of Ser, Gly, Ala, Pro, and the bis-DNP derivative of lysine from the other amino acid derivatives. It also separates DNP-isoleucine and DNP-leucine from the others, but does not distinguish between the two.

(11) *Biochem. J.*, **48**, 126 (1951).

(12) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1953).

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INTERCONVERSION AND DEGRADATION OF REDUCING SUGARS BY ANION EXCHANGE RESINS

Sir:

In the paper chromatogram of a hydrolysate originating from a partly methylated cellulose, a fairly strong spot corresponding to D-fructose was discovered. Since cellulose does not contain fruc-