depending in the main on hydrogen bonding, with or without steric hindrance by branched aliphatic side chains. The hindered reduction of the mercaptides of PCV actually constitutes an intermediate case, as we have found that the phenylmercuric mercaptides of ovalbumin are completely irreducible polarographically.

The ideas put forward here represent a compromise between chemical and physical theories which have been proposed to account for the "masking" of protein –SH groups, since it is postulated that a chemical link (hydrogen bonding) leads to steric hindrance by branched aliphatic side chains.

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF MEDICINE
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

RUTH E. BENESCH
REINHOLD BENESCH

RECEIVED JUNE 11, 1953

ON THE POLAROGRAPHIC REDUCTION WAVE OF DEHYDROASCORBIC ACID

Sir:

Since 1938[1] the polarographic oxidation wave of ascorbic acid has been well known among polarographists; however, the reduction wave of dehydroascorbic acid has not yet been found. So we attempted to obtain this reduction wave by using relatively high concentrations of dehydroascorbic acid in polarographic solutions.

For that purpose crystalline dehydroascorbic acid was prepared, using Pecherer's method.[2] Our polarographic experimental conditions were as follows:

Concn. of dehydroascorbic acid, 0.025∼ 0.1 *M*
*p*H range 2∼5 in McIlvaine's buffer solution
Temperature range 10∼50° (at 5° intervals)

On the other hand, we also used dehydroascorbic acid which was not crystallized but was prepared by oxidizing ascorbic acid in buffer solutions with equimolar amounts of iodine. Consequently these solutions became more acidic, then we neutralized them partly with some portion of 1 *N* NaOH and used them as polarographic solutions without excluding I^- ions. These I^- ions showed polarographic oxidation wave but did not disturb the reduction wave of dehydroascorbic acid. The final *p*H of the solution was measured by means of a glass electrode.

The reduction wave of dehydroascorbic acid was very small at room temperature (about $^1/_{1000}$ of the expected diffusion current of dehydroascorbic acid) and had all the typical characteristics of the kinetic current which was prominently examined and explained by several authors when they had experimented with formaldehyde[3,4] or aldoses.[5,6] That is, characteristics such as the fact that the wave height of dehydroascorbic acid remains constant, independently of the height of the mercury reservoir (Fig. 1) and the temperature coefficient of this wave height is extraordinarily large (Fig. 2).

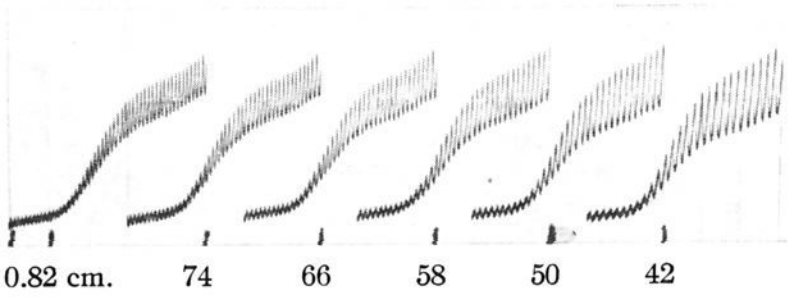

Fig. 1.—Constancy of the wave height of dehydroascorbic acid, independently of the height of the mercury reservoir: 0.1 mole of dehydroascorbic acid in McElvaine buffer solution, $S = ^1/_5$, *p*H 2.8, 25°.

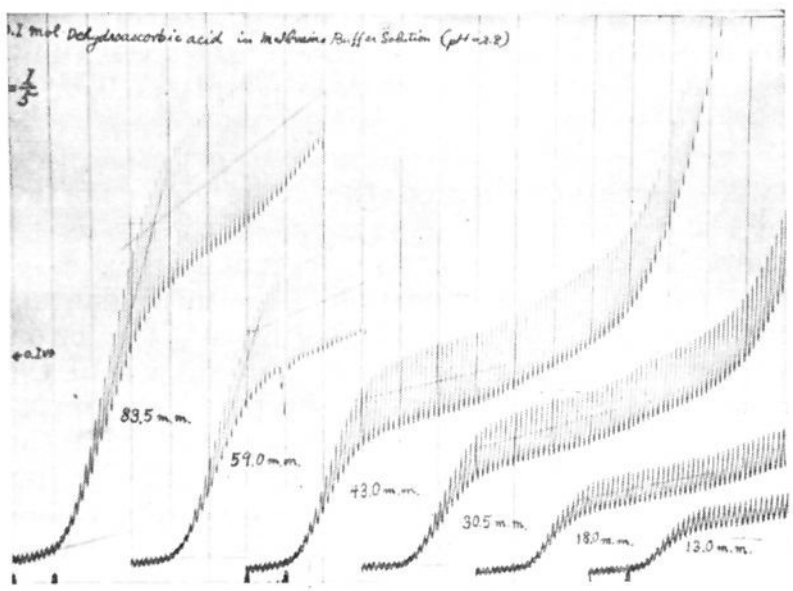

Fig. 2.—Increase of wave height of dehydroascorbic acid with increasing temperature: galv. sensitivity, 5.50×10^{-9} amp.; $m = 1.323$ mg./sec.; $t = 2.7$ sec./drop; 0.1 mole of dehydroascorbic acid in McElvaine buffer solution, *p*H 2.8; $S = ^1/_5$.

The above described results may be reasonably explained, if we assume that the electroactive form of dehydroascorbic acid (unhydrated form) is scarce in aqueous solutions and its limiting current is controlled practically by the rate of the dehydration of the hydrated dehydroascorbic acid. Also this assumption does not seem to conflict with others' reports in which they assumed that the equilibrium between hydrated and unhydrated dehydroascorbic acid would be much shifted toward the hydrated form.[7,8]

From the relationship between the temperature and the limiting current of the reduction wave we obtained the activation energy of the dehydration reaction of hydrated dehydroascorbic acid. The activation energy is about 13 kcal., which was proved to be independent of *p*H.

The fact that the reduction product of dehydroascorbic acid at the dropping mercury electrode is ascorbic acid was polarographically substantiated after the controlled potential electrolysis[9] of dehydroascorbic acid.

(1) E. Kodicek and K. Wenig, *Nature*, **142**, 38 (1938).
(2) B. Pecherer, THIS JOURNAL, **73**, 3827 (1951).
(3) K. Vesely and R. Brdicka, *Collection Czechoslov. Chem. Communs.*, **12**, 313 (1947).
(4) R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **30**, 706 (1947).
(5) R. Brdicka and K. Wiesner, *Collection Czechoslov. Chem. Communs.*, **12**, 138 (1947).
(6) P. Delahay and J. E. Strassner, THIS JOURNAL, **74**, 893 (1952).
(7) Z. Vavrin, *Collection Czechoslov. Chem. Communs.*, **14**, 367 (1949).
(8) R. Brdicka and P. Zuman, *ibid.*, **15**, 766 (1950).
(9) J. J. Lingane, *Trans. Faraday Soc. Discussion*, **1**, 203 (1947).

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.

LABORATORY OF BIO-PHYSICAL CHEMISTRY
FACULTY OF AGRICULTURE — SÔZABURO ONO
NANIWA UNIVERSITY — MASANOSUKE TAKAGI
SAKAI CITY, JAPAN — TAMOTU WASA

RECEIVED JULY 15, 1953

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).

W. A. LANDMANN
THE ARMOUR LABORATORIES — M. P. DRAKE
CHICAGO, ILLINOIS — W. F. WHITE

RECEIVED JUNE 22, 1953

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-