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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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).

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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-

tose it became of interest to learn at what stage of our experiment a partial transformation of the glucose moiety to fructose had taken place. During the working-up procedure of the acid hydrolysate use was made of a strongly basic anion exchange resin, Amberlite IRA-400 (OH form)[1], for neutralization. It seemed logical to assume that the conversion was caused by the catalytic action of the resin in a reaction reminiscent of the base-catalyzed Lobry du Bruyn transformation. This was borne out by the fact that when 25 ml. of a 2% glucose or fructose solution was allowed to stand in contact with 3 g. of the resin, the following analytical data were obtained by combination of the alkaline hypoiodite and the Somogyi methods.

TABLE I

Time, hr.	Starting glucose % Glucose	Starting glucose % Fructose	Starting fructose % Glucose	Starting fructose % Fructose
0	100	..	..	100
24	92.9	7.1	21.1	78.9
48	84.3	15.7	37.1	62.9
120	75.3	24.7	52.3	47.7
168	71.4	28.6	52.7	47.3
212	70.3	29.7	54.3	45.7

The percentages given above refer only to the carbohydrate remaining, since it was found that approximately 30% of the sugars was destroyed into acidic products. Acidic degradation of certain sugars was reported by Phillips and Pollard[2] and by Hulme,[3] who chromatographically detected, though not completely identified, at least five acidic residues.

These results made it desirable to determine what effect the resin has on other sugars. When cellobiose was allowed to remain in contact with the resin for 70 hours, there was detected, in addition to unreacted cellobiose, an unidentified disaccharide as well as a considerable quantity of glucose and fructose. The amount of glucose and fructose increased with time until after 238 hours most of the disaccharides disappeared. Similar results were obtained with maltose where the other disaccharide was assumed to be maltulose. When turanose was allowed to remain in contact with the resin for 46 hours, the only sugars that could be detected chromatographically were glucose and fructose. In all these disaccharide reactions there was an accompanying destruction of the carbohydrate to acidic products analogous to the glucose case. No reaction was observed with sucrose and several unidentified spots were obtained with D-arabinose, one of them presumably representing ribulose.

It also became of interest to learn what reaction, if any, occurred when the resin was in the carbonate rather than the hydroxyl form. Neither maltose nor glucose underwent any conversion after 48 hours. Turanose, however, was converted in considerable amount to glucose with the complete absence of fructose.

When glucose was treated with a weakly basic resin, Amberlite IR-4B,[1] there was detected, after 48 hours, a weak spot of fructose beside glucose on the chromatogram.

(1) Manufactured by Rohm and Haas Co., Philadelphia, Pa.
(2) J. D. Phillips, and A. Pollard, *Nature*, **171**, 41 (1953).
(3) A. C. Hulme, *Nature*, **171**, 610 (1953).

The mechanism of these reactions cannot be postulated on the basis of these preliminary experiments. However, it appears that the presently accepted ene-diol mechanism for the Lobry du Bruyn transformation does not hold in this case, as evidenced by the absence of mannose in the glucose–fructose interconversion.

These results bring to light two important considerations which should be accorded the strictest attention. First, extreme care must be exercised in using such ion exchange resins in conjunction with solutions of reducing carbohydrates. Careful analysis must be performed on the column effluent to determine whether any considerable resin-catalyzed reaction took place. Second, one must consider the use of these anionic resins as catalysts for facile interconversions of carbohydrates. The evident advantage of easy removal of catalyst from the reaction mixture makes such a possibility most attractive.

Investigations along these lines are being continued.

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RECEIVED AUGUST 3, 1953

THE CONVERSION OF L-LYSINE-6-C^{14} TO PIPECOLIC ACID IN THE RAT

Sir:

L-Pipecolic acid recently has been determined to be a constituent of certain plants.[1,2] This amino acid bears a close structural relationship to lysine, and while investigating the metabolism of the latter in rats, we have found evidence that L-lysine-6-C^{14} is converted in significant measure to radioactive pipecolic acid. The method used in making this observation was one which has been utilized successfully in this laboratory for detecting other specific catabolites of radioactive precursors. A solution containing 6.4 mg. of L-lysine-6-C^{14} monohydrochloride (3.50×10^8 disintegrations/min./mMole) and 500 mg. of L-pipecolic acid[3] was injected intraperitoneally into a male Wistar rat which had previously been fasted for 24 hours. The urine was collected for 24 hours, filtered and passed consecutively through columns of the ion exchangers Amberlite IR-4 and IRC-50. The effluent was evaporated to dryness, and the residue was converted to a copper salt by treatment with copper carbonate in 95% ethanol. After treatment of the copper salt with hydrogen sulfide in hydrochloric acid solution, the L-pipecolic acid was recovered as the hydrochloride from an ethanol–acetone mixture. Two recrystallizations yielded approximately 70 mg. of a material which showed only one spot on a ninhydrin treated paper chromatogram (collidine–lutidine–water). The spot corresponded to that obtained with authentic

(1) R. M. Zacharius, J. F. Thompson and F. C. Steward, THIS JOURNAL, **74**, 2949 (1952). See also N. Grobbelaar and F. C. Steward, *ibid.*, **75**, 4341 (1953).
(2) R. I. Morrison, *Biochem. J.*, **53**, 474 (1953).
(3) The authors wish to thank Dr. F. C. Steward of Cornell University for his generous gift of this compound.