O_8N_8S: C, 50.1; H, 6.30; N, 13.3, equiv. wt. 527. Found: C, 49.4; H, 6.55; N, 13.2: equiv. wt., 526). This new approach to glutaminyl peptides has also afforded an excellent synthesis of glutamine.

III was condensed with IV by the pyrophosphite method[15] to give tosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl - S - benzyl - L - cysteinyl - L - prolyl-L-leucylglycine amide, m.p. 235–236° dec. (calcd. for $C_{45}H_{66}O_{11}N_{10}S_2$: C, 54.8; H, 6.74; N, 14.2. Found: C, 54.4; H, 6.85; N, 13.8). The latter was treated with sodium in liquid ammonia followed by benzyl chloride to give V which was condensed similarly with protected dipeptide II[16,17] to give the desired nonapeptide derivative (I). Three hundred sixty milligrams of the latter in several batches was treated with sodium in liquid ammonia followed by aeration in dilute aqueous solution at *p*H 6.5. The combined product was assayed by the chicken depressor method,[18,19] and possessed a total of approximately 29,000 units of activity. The material was subjected to countercurrent distribution in *s*-butyl alcohol and acetic acid. The activity was found to be concentrated in a single peak ($K = 0.34$). The material in the peak tube was not distinguishable in potency from one of our best preparations of natural oxytocin, when these were assayed repeatedly against one another. The batched material as isolated had a somewhat lower activity.

The synthetic material possessed the expected oxytocic activity on the isolated rat uterus. Furthermore, the synthetic product was fully effective in stimulating labor in the human.[20] The synthetic material likewise possessed milk-ejecting activity in the human; approximately 1 γ of either the synthetic or natural material given intravenously to patients induced milk ejection in 20–30 seconds.[20]

The synthetic material possessed the specific rotation $[\alpha]^{21.5}D$ $-26.1 \pm 1.0°$ (*c* 0.53, water) compared to $[\alpha]^{22}D$ $-26.2°$ (*c* 0.53, water) for natural oxytocin. It formed an active flavianate derivative[4] identical in melting point and crystalline form (fine, silky needles) with that obtained from natural oxytocin. The amino acid composition of the synthetic material after hydrolysis as determined with the starch column,[21] expressed as molar ratios, was: leucine 1.00, isoleucine 1.00, tyrosine 0.83, proline 0.92, glutamic acid 0.91, aspartic acid 0.93, glycine 0.98, cystine 0.87 and ammonia 3.04. The distribution coefficients in both *s*-butyl alcohol–acetic acid and *s*-butyl alcohol–ammonia, and the electrophoretic mobilities on paper (*p*H 4.6 and *p*H 10.7) were the same for both the synthetic material and natural oxytocin. No differences were detected in the infrared patterns.

If the synthetic product truly represents oxytocin, which it does in so far as we have been able to ascertain, this would constitute the first synthesis of a polypeptide hormone. What effect slight changes in the structure of a compound of such complexity might have on chemical, physical and biological properties must be investigated.

VINCENT DU VIGNEAUD[22]
DEPARTMENT OF BIOCHEMISTRY CHARLOTTE RESSLER
CORNELL UNIVERSITY MEDICAL COLLEGE JOHN M. SWAN[23]
NEW YORK, N. Y. CARLETON W. ROBERTS
PANAYOTIS G. KATSOYANNIS[24]
SAMUEL GORDON[25]

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(15) G. W. Anderson, J. Blodinger and A. D. Welcher, THIS JOURNAL, **74**, 5309 (1952).

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(20) The milk-ejecting and uterine-contracting activities in the human were tested through the kindness of Professor R. Gordon Douglas, Dr. Kenneth G. Nickerson and Professor Roy W. Bonsnes of our Department of Obstetrics and Gynecology.

(21) S. Moore and W. H. Stein, *J. Biol. Chem.*, **178**, 53 (1949).

(22) Appreciation is expressed to the Lederle Laboratories Division, American Cyanamid Company, for a research grant which has aided greatly in this study. Acknowledgment is also made to Parke, Davis and Company, Armour and Company, and Eli Lilly and Company for placing at our disposal posterior pituitary material used as starting material for preparation of the purified oxytocin.

(23) Fulbright Scholar on Smith Mundt grant-in-aid, on leave from Wool Textile Research Laboratory, C.S.I.R.O., Australia.

(24) Fellow of State Scholarships Foundation of Greece.

(25) Public Health Service Research Fellow.

ENZYMATIC CLEAVAGE OF GLYCINAMIDE FROM VASOPRESSIN AND A PROPOSED STRUCTURE FOR THIS PRESSOR-ANTIDIURETIC HORMONE OF THE POSTERIOR PITUITARY

Sir:

The partial hydrolysis of performic acid-oxidized arginine-vasopressin and the determination of the structure of the resultant peptides has led to the postulation by Popenoe and du Vigneaud[1] of the sequence Cysteic.Tyr.Phe.Glu.Asp.Cysteic.[Pro, Arg, Gly],[2] for the oxidized hormone and the probable sequence $\overline{\text{CyS.Tyr.Phe.Glu.Asp.CyS.}}$[Pro,Arg,Gly] for vasopressin itself. Additional data were obtained by application of the Edman degradation to performic acid-oxidized vasopressin and by other degradative reactions.[3]

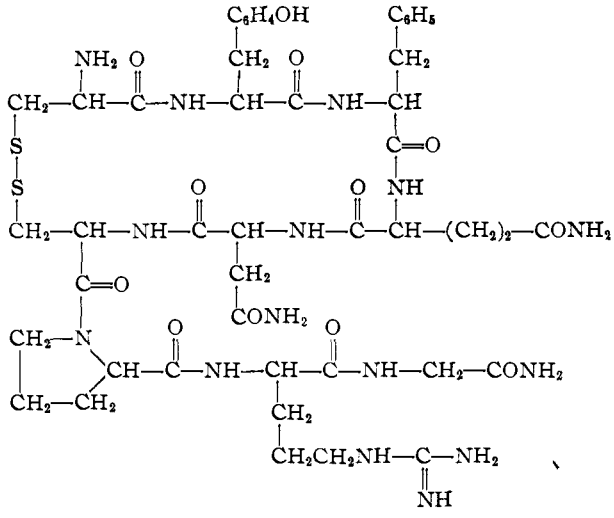
We have now obtained evidence, through enzymatic cleavage of vasopressin, that glycinamide is terminal and arginine is in the penultimate position, making possible the complete assignment of the sequence of amino acids in vasopressin. Assuming that the glutamic and aspartic acids occur as glutamine and asparagine residues (accounting for the other two of the three moles of ammonia in hydrolysates of vasopressin) we would therefore propose the following structure for arginine-vasopressin.

The enzymatic cleavage was obtained by incubation of arginine-vasopressin with trypsin for 6 hours at 38° and *p*H 7. The resultant fractions were separated and identified by two-dimensional paper chromatography. Two spots were obtained, one of which was identical with an authentic sample of glycinamide in its behavior on paper chroma-

(1) E. A. Popenoe and V. du Vigneaud, *J. Biol. Chem.*, in press.

(2) The convention is followed that when peptides of known structure are referred to, the residues are joined by a period and that in peptides of unknown sequence, the residues appear within brackets and are separated by commas. The amino acid appearing at the left in a known sequence is that bearing a free amino group.

(3) E. A. Popenoe and V. du Vigneaud, *J. Biol. Chem.*, **205**, 133 (1953).



tography. Hydrolysis of this fraction gave rise to glycine. The crystalline enzyme from *Aspergillus oryzae*,[4] also released glycinamide from arginine-vasopressin. The other fraction from the enzymatic hydrolysis was found to contain the other seven amino acids known to be present in vasopressin. This fraction gave a paper chromatographic spot close to the phenol solvent front, whereas the glycinamide showed an R_F of 0.66 in 75% phenol and 0.26 in butanol–acetic acid.

The components of a trypsin hydrolysate of arginine-vasopressin were then partially separated by countercurrent distribution between 2-butanol and 0.1% acetic acid. After 300 transfers the material was collected from those tubes in which glycinamide might be expected. The main component behaved in a fashion identical with glycinamide on the starch column with 2:1 propanol–0.5 *N* HCl as the developing agent. A sample was hydrolyzed and analyzed by starch column chromatography and glycine and ammonia were found in approximately equimolar proportions along with traces of other amino acids. The remaining material from the distribution contained the other seven amino acids of vasopressin in approximately equimolar amounts and only a small amount of glycine. Incubation of this fraction with arginase resulted in liberation of some urea, whereas arginase has no action on the intact hormone under the same conditions.

Since only those peptide bonds involving the carboxyl group of lysine or arginine are known to be hydrolyzed by trypsin,[5] the liberation of glycinamide by trypsin from vasopressin indicates the sequence arginylglycinamide.

It has been shown that lysine-vasopressin[6] has the same composition as arginine-vasopressin with the exception that it contains lysine instead of arginine. The trypsin hydrolysis of lysine-vasopressin has also yielded glycinamide. In view of the similarity in biological behavior of the two vasopressins, it would seem logical that the proposed structure for arginine-vasopressin represents also that of lysine-vasopressin,[7] with lysine replacing the arginine.

DEPARTMENT OF BIOCHEMISTRY VINCENT DU VIGNEAUD[8]
CORNELL UNIVERSITY MEDICAL COLLEGE
H. CLAIRE LAWLER
NEW YORK, NEW YORK EDWIN A. POPENOE

RECEIVED SEPTEMBER 18, 1953

(4) W. G. Crewther and F. G. Lennox, *Nature*, **165**, 680 (1950).

(5) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1950).

(6) E. A. Popenoe, H. C. Lawler and V. du Vigneaud, THIS JOURNAL, **74**, 3713 (1952).

(7) A synthesis of the octapeptide structure here proposed for lysine-vasopressin has led to biologically active material (V. du Vigneaud, E. A. Popenoe and R. Roeske, unpublished data). The synthesis was parallel to the synthesis of oxytocin (V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, THIS JOURNAL, **75**, 4879 (1953)), with ε-tosyllysine replacing leucine and phenylalanine replacing isoleucine in the series of reactions. The crude reaction product of the final step possessed pressor and antidiuretic activity. The work is being continued.

(8) Appreciation is expressed to the Lederle Laboratories Division, American Cyanamid Company, for a research grant which has aided greatly in this study. Acknowledgment is also made to Parke, Davis and Company and Armour and Company for placing at our disposal posterior pituitary material used as starting material for preparation of the purified vasopressin. We are also grateful to Dr. F. G. Lennox of the Wool Textile Research Laboratory, C. S. I. R. O., Australia, for a gift of the crystalline mold enzyme.

CARBON ISOTOPE CONSTITUTION OF SOME ACETIC ACIDS

Sir:

In the course of a reinvestigation[1] of the carbon isotope effect in the decarboxylation of malonic acid, product acetic acid was to be degraded, by a modification of Phares' application[2] of the Schmidt reaction,[3] to methylamine and carbon dioxide. During development of the modification, trial analyses were made of various commercial reagent grade acetic acids. It was found that there was considerable variation in their carbon isotope constitution.

Through the coöperation of the Carbon and Carbide Chemicals Co. we obtained a sample of glacial acetic acid made at their Niagara Falls plant by the sequence ethylene → ethanol → acetaldehyde → acetic acid. The results of three determinations of the carbon isotope ratios (C^{13}/C^{12}) of carbon dioxide obtained from methylamine (R_M) and from total combustion of the acetic acid (R_D) were: $R_M = 0.010793 \pm 0.000002$; $R_D = 0.010789 \pm 0.000001$. Values obtained experimentally for the carbon isotope ratio of the original carboxyl carbon (R_C) were within a few tenths of a per cent. of that expected from the two just recorded, but were subject to much larger variation. It is believed that this spread was due to erratic inclusion of extraneous carbonate in small amount in the carbonate obtained from the degradation. It seems preferable to calculate R_C from R_M and R_D, whence $R_C = 0.010785 \pm 0.000002$, and $R_M - R_C = 0.000008 \pm 0.000002$. Evidently the symmetry of the ethylene molecule is destroyed in a step which does not distinguish between light and heavy ends of the molecule, and the subsequent reactions are sufficiently quantitative to preclude net isotope fractionation in the product.

The Celanese Corporation of America kindly supplied samples of glacial acetic acid and precursor acetaldehyde, materials made by the air oxidation

(1) P. E. Yankwich and A. L. Promislow, in preparation.

(2) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951).

(3) K. F. Schmidt, *Ber.*, **57B**, 704 (1924).