times (after 250, 450, 650 and 1080 transfers) to replace with fresh solvent the solutions of the tubes containing material with no pressor activity. The solutions from 35 tubes containing the active material were pooled. The organic phase was extracted three times with water and the extracts were combined with the main aqueous phase. This solution was passed through a column of Amberlite IR-45 in the acetate form to remove the *p*-toluenesulfonic acid. The solution was then concentrated in a rotary evaporator and lyophilized to 120 mg. of a powder which had an activity of approximately 250 pressor units/mg.

This material was subjected to electrophoresis on a cellulose block[17,23] at *p*H 4.0 in a pyridine–acetate buffer for 42 hours at 5° with a potential of 400 volts. The most active fraction, weighing 38 mg., possessed 360 to 440 units/mg. of pressor activity on assay in the rat.[16]

Comparison of Synthetic and Natural Arginine Vasopressin.—The partition coefficients of the synthetic material as determined by countercurrent distribution in the systems 2-butanol–0.06 *M* *p*-toluenesulfonic acid and 2-butanol–0.1% acetic acid (*K*'s of 0.85 and 0.11, respectively) agreed within the deviations expected experimentally with the partition coefficients of natural arginine-vasopressin in the same solvent systems (*K*'s of 0.87 and 0.11,[24] respectively).

Samples of synthetic and natural arginine-vasopressin were placed side by side on a strip of Whatman No. 1 filter paper and subjected to paper electrophoresis[25] at *p*H 5.6 and at *p*H 4.0 in pyridine–acetate buffer. The synthetic product had a mobility identical with that of the natural product and migrated as a single component at both *p*H's.

A sample of the synthetic material was chromatographed[26] on an Amberlite IRC-50 column with an ammonium acetate buffer of *p*H 6.4. The synthetic product migrated on the column at the same rate as a sample of natural arginine-vasopressin chromatographed under identical conditions. No indication of the presence of more than one component was obtained under the experimental conditions used.

Amino acid analysis of the synthetic material after hydrolysis by the starch column chromatographic method[27] showed the expected amino acids and ammonia to be present in the following molar ratios, with the ratio for phenylalanine arbitrarily taken as 1: phenylalanine 1.00, tyrosine 0.88, proline 0.84, glutamic acid 1.06, aspartic acid 0.86, glycine 1.03, arginine 0.95, cystine 0.83 and ammonia 2.90.

Acknowledgments.—The authors are indebted to Mrs. Sylvia Kirsimagi White and Miss Gertrud Graubart for carrying out the assays for pressor and avian depressor activity and to Mrs. Lorraine S. Abrash for the amino acid analyses on the starch column.

(23) "Solka-Floc," a cellulose product of Brown Co. of New York, N. Y., was used.

(24) This value was determined by Dr. Peter G. Condliffe in this Laboratory.

(25) H. G. Kunkel and A. Tiselius, *J. Gen. Physiol.*, **35**, 89 (1951).

(26) The ion-exchange chromatography was performed by Dr. Albert Light of this Laboratory, to whom we wish to express our thanks.

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

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The N-Terminal Sequence of the β Chains[1] of Normal Adult Human Hemoglobin

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The N-terminal sequence of the β chains of normal adult human hemoglobin is val-his-leu. Kinetic studies of the rate of hydrolysis of the val-his bond in the form of di-DNP-val-his-leu show that the hydrolysis is accompanied by a reaction of an unknown kind. The hydrolytic behavior at the N-termini is different for the α and the β chains of DNP-globin, but it has not been ascertained whether this difference is one of rate of hydrolysis or of destruction of the N-terminal amino acid in the course of the hydrolysis.

Introduction

Recent quantitative experiments[2] have led to the conclusion that normal adult human hemoglobin has four N-terminal valyl residues and that two kinds of N-terminal sequences are present. Although it was ascertained readily that two chains (termed the α chains[1]) release DNP-val-leu rapidly when DNP-globin is hydrolyzed, no N-terminal peptides that originated in the other chains (the β chains[1]) could be definitely identified at that time. This paper describes the isolation and identification of di-DNP-val-his-leu from the N-terminus of the β chains and a study of the kinetics of the hydrolysis of this peptide and of di-DNP-val-his.

Experimental

Preparation of DNP-globin.—Samples of DNP-globin were prepared by the methods previously described[2,3] except that in some preparations the *p*H was maintained at 8.15 instead of 9.0 during the dinitrophenylation. This change did not affect the final product.

Partial Hydrolysis of DNP-globin.—The procedure of partial hydrolysis was that previously described.[2] Samples ranged in size from 0.2 to 1.0 g., and the proportion of acid to weight of sample was also the same as previously used.

Extraction of the Partial Hydrolysates.—DNP-Valine and DNP-val-leu may be extracted easily with ether from a partial hydrolysate but the N-terminal peptides of the β chains are extracted with difficulty even with ethyl acetate. The following extractive procedure is the most satisfactory that has been devised in the course of these experiments.

The following quantities are adequate for the extraction of the hydrolysate of 0.2 g. of DNP-globin and should be increased proportionally for larger samples. After the hydrolysate had been transferred to a separatory funnel with 10 ml. of 6 *N* hydrochloric acid, the DNP-valine, DNP-val-leu and dinitroaniline (released from the α chains) were extracted with 5 × 25 ml. of ether. The procedure thus far is essentially that of ref. 2; if separation of the compounds of the ether extract is desired, the methods described in ref. 2 may be used. The hydrolysate was then extracted with 15 × 10 ml. of ethyl acetate in order to remove the N-terminal peptides from the β chains. Finally, the ethyl acetate extract was washed with 10 × 20 ml. of water to each portion of which 6 drops of 6 *N* hydrochloric acid was added. After evaporation of the ethyl acetate, the residue of peptides was ready for chromatography.

The extraction may be modified somewhat, depending on the purposes of the experiment. Because 65 to 75% of the

(1) Formerly called the B chains.[2] After discussion with Dr. Vernon Ingram, it was decided that if the polypeptide chains of normal adult human hemoglobin were designated α and β instead of A and B, there would be less likelihood of confusion with the names of the hemoglobins themselves, that is, hemoglobin A, S, C, etc.

(2) H. S. Rhinesmith, W. A. Schroeder and L. Pauling, THIS JOURNAL, **79**, 4682 (1957).

(3) H. S. Rhinesmith, W. A. Schroeder and L. Pauling, *ibid.*, **79**, 609 (1957).

peptides may be extracted in the first five extractions with ethyl acetate, this amount of extraction is adequate for isolative work. During extraction with ethyl acetate the phases separate slowly, and after a number of extractions there may be no separation until a few ml. of water has been added. Ethyl acetate removes not only the N-terminal peptides of the β chains but also ε-DNP-lysyl peptides. A large portion of the latter is removed by washing with water.

Chromatographic Isolation of the N-Terminal Peptides from the β Chains.—The chromatographic methods of Green and Kay[4] for DNP-amino acids as extended to DNP-peptides by Schroeder and Honnen[5] were, in general, followed for the isolation of the peptides. The chromatographic procedure that is required to isolate the N-terminal peptides of the β chains is more cumbersome than that required for those of the α chains.[2] A first chromatogram was used mainly to separate the N-terminal DNP-peptides from ε-DNP-lysyl peptides that were not washed from the ethyl acetate extract. The N-terminal DNP-peptides were then rechromatographed in order to separate them.

The residue that remained after the evaporation of the ethyl acetate was dissolved in the minimum amount of 20AA–20A-B[6] and chromatographed on a 13 × 150-mm. column of silicic acid–Celite first with 3 *V*[6] ml. of 3AA–15A–L and then with 6 *V* ml. of 4AA–20A–L as developer. The ε-DNP-lysyl peptides were strongly fixed on this chromatogram, while the N-terminal DNP-peptides moved in a rather ill-defined zone through the column and were partially washed into the filtrate. The portion of the N-terminal peptides that remained on the column was eluted with 1:4 ethanol–ether, combined with the portion in the filtrate and prepared for rechromatography. The sample solvent in the above chromatogram is required to dissolve the ε-DNP-lysyl peptides but is so strong a developer for the N-terminal peptides that the latter do not form well-defined zones. Consequently, for the rechromatography a minimum amount (generally 2 ml.) of 10AA–20A–L was used as a sample solvent and the chromatogram on a 9 × 150-mm. column was developed as above. At the end of the chromatogram, two well-defined and fairly well separated zones are present on the column: the one located between 50 and 85 mm. from the top of the column is a dipeptide and the other between 110 mm. and the bottom of the column and partially in the filtrate is a tripeptide. Elution was then made as above.

Identification of the N-Terminal DNP-Peptides.—The qualitative and quantitative amino acid composition of the peptides was determined after complete hydrolysis in refluxing 6 *N* hydrochloric acid. The N-terminal DNP-amino acid was extracted from the hydrolysate, the non-N-terminal amino acids were dinitrophenylated and the DNP-amino acids were identified chromatographically by the method of Green and Kay[4] and estimated spectrophotometrically. In general, the methods previously described[7] for the identification of DNP-peptides were followed.

The method of Green and Kay[4] does not provide for the identification of di-DNP-histidine. Although di-DNP-histidine is more strongly adsorbed than the DNP-amino acids of Group I,[4] its extractive behavior is such that the scheme of Green and Kay need not be revised to include it. Extraction of an acidic solution of a mixture of DNP-amino acids with ether will remove those DNP-amino acids that may be separated by the method of Green and Kay, but di-DNP-histidine is not extracted. Subsequent extraction with ethyl acetate will remove di-DNP-histidine and the presence of the latter may then be confirmed chromatographically. Although development first with 3 *V* ml. of 3AA–15A–L and then with 6 *V* ml. of 4AA–20A–L is the most satisfactory chromatographic procedure for di-DNP-histidine, unfortunately, its chromatographic behavior is almost identical with that of the dipeptide mentioned above. Positive identification requires rehydrolysis in order to ascertain that the DNP-peptide is absent.

Spectrophotometry.—When a single DNP-group is present in a DNP-amino acid or DNP-peptide and is attached to an α-amino group, the spectra of the DNP-compounds in glacial acetic acid are essentially identical[8] and have maxima at about 340 and 260 mμ and a minimum at about 288 mμ. Furthermore, the ratio of the absorption of the main maximum at 340 mμ to the absorption at the minimum is about 6 and the ratio of the absorptions at the maxima is about 1.8. The spectra of di-DNP-histidine and di-DNP-histidyl peptides are distinctly different. One maximum is observed at 340 mμ and a minimum is found at about 293 mμ; the ratio of absorptions of maximum to minimum is about 2.3. In addition, the absorption at 260 mμ is greater than at the maximum. The molecular extinction coefficient of di-DNP-histidine was determined to be 1.69×10^4 liters per mole cm. (1.61×10^4 for α-DNP-amino acids), and this value was used to calculate the amounts of di-DNP-histidyl peptides.

Partial Hydrolysis of Di-DNP-val-his and Di-DNP-val-his-leu.—Quantities of di-DNP-val-his and di-DNP-val-his-leu were isolated on a larger scale from 15-min. partial hydrolysates in refluxing 6 *N* hydrochloric acid. Portions of one or both peptides were then individually hydrolyzed in refluxing 6 *N* hydrochloric acid for periods of 1, 2, 4, 8, 16 or 22 hr. DNP-Valine was extracted from the hydrolysate with ether and unchanged peptide with ethyl acetate. Likewise, after the non-N-terminal products had been dinitrophenylated, the DNP-leucine was extracted with ether and the di-DNP-histidine with ethyl acetate. All the DNP-compounds were chromatographed and then determined spectrophotometrically.

Results and Discussion

The object of these experiments was to isolate and identify a peptide or peptides derived from the N-terminus of the β chains of normal human hemoglobin and to study the kinetics of the hydrolysis of such peptides. Exploratory experiments showed that the ethyl acetate extract of a 15-min. hydrolysate of DNP-globin in refluxing 6 *N* hydrochloric acid contained most of the N-terminal products derived from the β chains. Consequently, 15-min. hydrolysates have been used in this work and no attempt has been made to follow the increase and decrease of the N-terminal products of the β chains in the same way as the α chains have been studied previously.[2]

Identification of the DNP-Peptides from the N-Terminus.—Only two peptides in the ethyl acetate extracts contain N-terminal DNP-valine and hence are derived from the N-termini: their isolation has been described above. The more strongly adsorbed zone on complete hydrolysis was shown to contain N-terminal DNP-valine and an equimolar amount of di-DNP-histidine (after dinitrophenylation of the non-N-terminal amino acids): the peptide then is di-DNP-val-his. Likewide, by means of complete and partial hydrolysis, the less strongly adsorbed zone was identified as di-DNP-val-his-leu. The spectrum of the compounds is the only evidence for the statement that they are di-DNP-derivatives, that is, that the imidazole ring of the histidyl residue is dinitrophenylated.

Quantities of the N-Terminal Peptides in 15-Min. Hydrolysates.—In 15 min. of hydrolysis in refluxing 6 *N* hydrochloric acid, essentially all N-terminal DNP-valine is to be found either free or in peptide form in the ether or ethyl acetate extracts: only traces of DNP-valine are released if the extracted hydrolysate is further hydrolyzed. The α chains release DNP-val-leu as the N-terminal product almost quantitatively in the first 15

(4) F. C. Green and L. M. Kay, *Anal. Chem.*, **24**, 726 (1952).

(5) W. A. Schroeder and L. R. Honnen, THIS JOURNAL, **75**, 4615 (1953).

(6) Abbreviations for solvents and developers follow Green and Kay.[4] Thus, AA is for acetic acid, A for acetone, L for ligroin, B for benzene, etc., and 20AA–20A-B denotes 20 volume % AA and 20 volume % A in B.

(7) W. A. Schroeder, L. M. Kay, J. LeGette, L. Honnen and F. C. Green, THIS JOURNAL, **76**, 3556 (1954).

(8) W. A. Schroeder, *ibid.*, **74**, 5118 (1952).

min. of hydrolysis,[2] and it is apparent that the β chains likewise release the N-terminal products in extractable form within this period of time. A 0.1-g. sample of DNP-globin contains 1.14 μmoles of DNP-globin and hence 2.28 μmoles of both the α and β-chains. A 15-min. hydrolysate of 0.1 g. contains 0.1 μmole of DNP-valine, 2.0 μmoles of DNP-val-leu from the α-chains and 0.4 μmole of di-DNP-val-his and 1.1 μmoles of di-DNP-val-his-leu from the β chains. Clearly, the release of DNP peptides from the β chains is not as straightforward or as quantitative as is that from the α chains. The peptide bond between the second and third amino acid residues of the α chains must be very labile in comparison to its neighbors in order for such quantitative breaking to occur. On the other hand, the bonds near the N-terminus of the β chains are more similar in lability and, therefore, two peptides may be isolated. That the less quantitative results from the β chains are an expression of the structure is shown by study of the partial hydrolysis of the di- and tri-peptides.

Partial Hydrolysis of Di-DNP-val-his and Di-DNP-val-his-leu.—The results of the partial hydrolytic experiments with the N-terminal peptides are presented in Table I. They are in sharp contrast to those that have been obtained with DNP-val-leu.[2] When a sample of DNP-val-leu is partially hydrolyzed, the sum of the unhydrolyzed peptide and the DNP-valine released is essentially equivalent to the amount of starting material. When di-DNP-val-his-leu is hydrolyzed for one hr., only about 60% of the starting material may be accounted for in the form of DNP-valine, di-DNP-val-his and di-DNP-val-his-leu.[9] This hour of hydrolysis almost completely hydrolyzes the his-leu bond so that the expected N-terminal products are mainly DNP-valine and di-DNP-val-his. Because of low recovery, it is evident that some unrecognized product also is formed. This unrecognized product must contain N-terminal valine because the recovery of DNP-valine free or in peptide form improves as the period of hydrolysis is increased. Because this product does release DNP-valine, one would expect it to be yellow and easily detectable. Thus, it should be in the extracts because ether and ethyl acetate remove all the yellow color from a partial hydrolysate. The ether extract contains only DNP-valine: the ethyl acetate extract contains di-DNP-val-his (and di-DNP-val-his-leu in short hydrolyses of the tripeptide) as well as very strongly adsorbed orange-colored material. The latter, however, does not yield more than a trace of DNP-valine on further hydrolysis. If the unrecognized product were produced during the hydrolysis simply by removing the DNP-group from the imidazole ring of the histidyl residue, dinitrophenylation of the extracts of a hydrolysate would reform the di-DNP-compound and result in a better recovery: when the extracts were dinitrophenylated, no greater amount of di-DNP-val-his was isolated. It may be concluded, therefore, that the degradation during hydrolysis follows a different course and perhaps is associated with an opening of the imidazole ring in a manner somewhat analogous to the opening of the ring in DNP-proline that was observed by Scanes and Tozer.[10]

(9) The data in parentheses in Table I were obtained consecutively but at a later date than all other data. There was no difference in experimental procedure and we are unable to explain the disagreement in the two sets of values for di-DNP-val-his at one hr. of hydrolysis.

TABLE I

COURSE OF THE HYDROLYSIS OF DI-DNP-VAL-HIS AND DI-DNP-VAL-HIS-LEU IN REFLUXING 6 *N* HYDROCHLORIC ACID[a]

Time, hr.	DNP-valine, μmoles (cor.)[b] Individual values	Av.	Di-DNP-val-his, μmoles (cor.)[c] Individual values	Av.	% of N terminal DNP-valine recovered[d]
1	0.15, 0.14, 0.16 (0.14[e], 0.14)[f]	0.15	0.42, 0.34, 0.39 (0.54,[e] 0.64)[f]	0.38	53[g]
2	0.22	.22	Not determined	..	..
4	0.26, 0.45 0.34,[e] 0.35	.35	0.41, 0.31[e], 0.27	.33	68
8	0.52[e]	.52	0.22[e]	.22	74
16	0.82	.82	.08	.08	90
22	0.82,[e] 0.93 0.87, 0.87	.87	.04[h]		91[h]

[a] These experiments were made with 1 to 3 μmoles of di- or tripeptide and all results were recalculated to a starting quantity of 1.00 μmole. [b] Chromatographic correction of 2.5% per chromatogram plus hydrolytic correction of 8% per 22 hr. or an appropriate fraction for shorter times (see ref. 2). [c] Chromatographic correction of 7% per chromatogram only. [d] Based on the sum of the average μmoles DNP-valine plus di-DNP-val-his recovered compared to the μmoles of starting peptide. [e] The dipeptide was the starting material in these instances. [f] See Footnote 9. Not included in average. [g] About 5% of the tripeptide is unhydrolyzed after one hr. and, hence, must be added to this figure to give the total DNP-valine recovered. [h] Only when a 3-μmole sample of the peptide was used and a careful search was made was it possible to detect what was assumed to be the dipeptide.

At periods of hydrolysis longer than one hr., the quantity of leucine that may be isolated is essentially equivalent to the quantity of starting peptide. A recovery of only about 65% of the histidine in one form or another is observed throughout the entire course of the hydrolysis. This result may in part be due to incomplete dinitrophenylation of the histidine in our procedure to determine the non-N-terminal amino acids. Experiments with the dinitrophenylation of histidine have given only about 60% dinitrophenylation; these results accord with the report of Akabori and collaborators[11] to the effect that histidine is not quantitatively dinitrophenylated.

When the data of Table I are plotted, certain facts are evident. If the side reactions to form unrecognized product had continued at the rate that is apparent during the first hour of hydrolysis, little or no di-DNP-val-his would have been detected after 2 hr. of hydrolysis. Such is not the case. After the first hour of hydrolysis, the di-DNP-val-his decreases rather slowly and a few % seem to survive 22 hr. of hydrolysis. Thus, conditions must be very different in the first hour of hydrolysis compared to what they are later. Although during this first hour the his-leu bond is essentially completely hydrolyzed, it is unlikely that the hydrolysis of this bond influences the conversion to the unrecognized product because the results are the same regardless of whether the di- or

(10) F. S. Scanes and B. T. Tozer, *Biochem. J.*, **63**, 282 (1956).
(11) S. Akabori, *et al.*, *Bull. Soc. Chem. Japan*, **29**, 507 (1956).

tri-peptide is hydrolyzed. It may be that the products of hydrolysis have an inhibitory effect on the further conversion or possibly, but improbably, there is an equilibrium between the di-DNP-val-his and the unrecognized product.

Conclusions

Our earlier experiments[2] on the release of the N-terminal products of DNP-globin showed the almost immediate release of DNP-val-leu from the α chains. Then, simultaneously, there followed the hydrolysis of the val-leu bond and of an unknown bond from the β chains: the hydrolysis of both bonds yielded DNP-valine. Inasmuch as the hydrolysis of the val-leu bond was a straightforward, uncomplicated reaction for which a first-order reaction rate constant could be obtained, it was possible to calculate a reaction rate constant for the hydrolysis of the unknown bond. On the basis of this calculated constant, the β chains release DNP-valine more slowly than does the DNP-val-leu from the α chains and, indeed, 20% of each β chain should still be unhydrolyzed after 22 hr. in refluxing 6 *N* hydrochloric acid. If 20% of each of the two β chains is indeed unhydrolyzed after 22 hr., it provides a reasonable explanation of our earlier finding that DNP-globin contained 3.6 N-terminal valyl residues[3]: 20% of two chains equals 0.4 N-terminal residue and thus the actual number of N-terminal residues would be four.

Because this kinetic interpretation of the results is difficult to correlate with the known rates of hydrolysis of peptide bonds, serious consideration had to be given to the possibility that the rate constant was an apparent one only and that actually the destruction of DNP-valine in the course of the hydrolysis of the β chains was greater than the destruction of DNP-valine in the course of the hydrolysis of the α chains. These possibilities have been discussed in detail in previous publications.[2,3]

The present kinetic study of the hydrolysis of the DNP-peptides from the β termini has failed to provide an unequivocal answer to these questions. Because of the side reactions that occur during the hydrolysis of di-DNP-val-his and di-DNP-val-his-leu, the calculation of a reaction rate constant is meaningless. Comparison, therefore, cannot be made with the constant for the β chains that was calculated from the hydrolysis of DNP-globin.[2] If the constant is correct, 20% of di-DNP-val-his should be unhydrolyzed after 22 hr. in refluxing 6 *N* hydrochloric acid. Actually, at most, only a few per cent. may be detected. One might, therefore, conclude that the constant for the hydrolysis of the β chains is very similar to that of DNP-val-leu of which about 5% is unhydrolyzed after 22 hr. This conclusion is unwarranted because of the complicated course of the hydrolysis of di-DNP-val-his and because of our ignorance as to whether or not unrecognized products may still be present after 22 hr. On the other hand, if both the di-DNP-val-his and the unrecognized product are essentially hydrolyzed in 22 hr., the recovery of only 85 to 90% of the DNP-valine must mean that the destruction of DNP-valine is different in the course of the hydrolysis of the two chains.

One can conclude only that our original difficulty in arriving at an integral number of N-terminal residues in DNP-globin is associated with the different hydrolytic behavior of the two types of chains, but it cannot be decided with certainty whether the difference lies in the rates of hydrolysis or in the degree of destruction.

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PASADENA, CALIFORNIA

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT AND OCEANOGRAPHIC INSTITUTE OF THE FLORIDA STATE UNIVERSITY]

The Characterization of Polyaspartic Acid and Some Related Compounds[1,2]

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The "polyaspartic acid" obtained by heating aspartic acid above the boiling point of water is shown to be a mixture consisting of polyimide molecules with tightly held water in a ratio of almost one molecule of water per imide linkage. This water is removable by rigorous drying under reduced pressure at 200°. The polyimide polyhydrate can be converted to a true peptide-type polyaspartic acid by brief warming with dilute sodium hydroxide. The relationships between these various species have been clarified by study of the infrared analyses and in other ways. A typical average molecular weight for polyaspartic acid is 11,000, as determined by end-group assay. Data are presented to show that slightly hydrolyzed diketopiperazines may yield in chemical analyses results which resemble closely those from linear peptides. Rapid methods for distinction between diketopiperazines and polyamino acids were developed and are evaluated. These studies showed that the polymer is definitely not a diketopiperazine.

Syntheses of polypeptides and other protein-like materials in the past have included stepwise procedures,[3] fabrication of materials with recurrent amide linkages as exemplified by nylon[4] and the conversion of amino acids to "polyamino acids" by various methods.[1,5–7] For the most part, poly-

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(2) Except for the title, the term polyaspartic acid is not used without qualification in this paper until the evidence for the prevalent structures is reviewed.

(3) E. Abderhalden and A. Fodor, *Chem. Ber.*, **49**, 561 (1916).

(4) W. H. Carothers, U. S. Patent 2,071,250 (1937).

(5) E. Katchalski, *Advances in Protein Chem.*, **6**, 123 (1951).

(6) R. R. Becker and M. A. Stahmann, *J. Biol. Chem.*, **204**, 737 (1953).

(7) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956.