Table II—Probability of Meeting Content Uniformity Requirements

γ_p	γ_y 0.01	0.03	0.05
	when $\delta_p = -0.04$, $\delta_y = 0.02$		
0.01	1.0000	1.0000	0.9954
0.03	1.0000	0.9997	0.9663
0.05	0.9896	0.9539	0.7871
	when $\delta_p = 0.09$, $\delta_y = -0.08$		
0.01	1.0000	1.0000	0.9986
0.03	1.0000	1.0000	0.9786
0.05	0.9836	0.9459	0.7943
	when $\delta_p = 0.07$, $\delta_y = -0.01$		
0.01	1.0000	0.9986	0.7794
0.03	0.9996	0.9560	0.6310
0.05	0.8593	0.6823	0.4063

101 mg./tablet with coefficients of variation of 3% for P and 4% for Y, then the probability of meeting the content uniformity requirements as specified in USP XVII and NF XIII is 0.8283.

Although it is not feasible to publish complete tables of $P[\pi]$ for even a representative indexing of γ_p, γ_y, δ_p, and δ_y, a few additional examples (Tables I and II) are included.

DISCUSSION

Recalling that the probabilities of meeting content uniformity requirements depend only upon the four parameters δ_p, δ_y, γ_p, and γ_y, it is convenient to note that these probabilities are symmetric with respect to the pairs (δ_p, γ_p) and (δ_y, γ_y). That is, if $(\delta_p, \gamma_p) = (0.07, 0.03)$ and $(\delta_y, \gamma_y) = (-0.01, 0.05)$, then the probability of acceptance is the same as if $(\delta_p, \gamma_p) = (-0.01, 0.05)$ and $(\delta_y, \gamma_y) = (0.07, 0.03)$. This can be easily verified from Eqs. 6 and 7.

The IBM Mathpack Subroutine NDTR (12) was used to calculate the function $F[\,]$. Since this particular subroutine has a maximum error of $7(\times 10^{-7})$, the computed probabilities are in general more accurate than if they were computed using $F[\,]$ from usual tabled sources of the cumulative normal distribution. The difference is in the fourth decimal place and of little practical significance.

Note that several probabilities in Tables I and II are given as 1.0000. This means that, even though it is not absolutely certain that a lot will meet content uniformity requirements under the indexed parameter conditions, the probabilities of acceptance are so high that rounding to four decimal places carries these values to 1.0000.

The formulas in this paper compute the probability of meeting content uniformity requirements for given population parameters γ_p, γ_y, δ_p, and δ_y. They characterize the content uniformity sampling plan and are to be used as supplemental information in making decisions before and during the production of particular products.

REFERENCES

(1) J. P. Comer, H. L. Breunig, D. E. Broadlick, and C. B. Sampson, *J. Pharm. Sci.*, **59**, 210(1970).

(2) C. Roberts, *Technometrics*, **11**, 161(1969).

(3) L. A. Goodman, *J. Amer. Statist. Ass.*, **55**, 708(1960).

(4) C. C. Craig, *Ann. Math. Statist.*, **7**, 1(1936).

(5) L. A. Aroian, *ibid.*, **18**, 265(1947).

(6) R. S. DeZur and J. D. Donahue, *Aeronaut. Res. Lab. Tech. Rep.*, **1965**, 71.

(7) I. W. Burr, Chemical Division, American Society for Quality Control, *News* (*Special Issue*), **10**, 7(1964).

(8) C. B. Sampson, "Statistical Procedures for the Estimation of Content Uniformity," presented to the APhA Academy of Pharmaceutical Sciences, Pharmaceutical Analysis and Control Section, Washington, D. C. meeting, November 1969.

(9) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 905.

(10) "National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 799.

(11) "Handbook of Tables for Probability and Statistics," W. N. Beyer, Ed., The Chemical Rubber Co., Cleveland, Ohio, 1966.

(12) IBM System/360 Scientific Subroutine Package (360A-CM-03X) Version III, Program H20-0205-3, International Business Machines, Technical Publications Dept., White Plains, NY 10601

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Solid-Phase Synthesis and Degradation of a Model Polypeptide by an Automated Approach

ANDREW M. TOMETSKO*, JOHN TISCHIO, and JOHN GARDEN, II

Abstract □ The solid-phase synthesis and degradation of a model polypeptide, L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-alanine, was carried out using a computer-oriented automated approach. Two computer programs were used to generate control paper tapes for the total synthesis and degradation of the model peptide. Encouraging results were obtained in automating the degradation of polypeptides on the solid phase and in carrying out the degradation in a nonaqueous solvent system. The course of the synthesis and the degradation was monitored by amino acid analysis of acid hydrolyzates of the resin which were taken periodically. The automated solid-phase degradation of naturally occurring polypeptides and proteins should be possible through modifications of the described approach.

Keyphrases □ Polypeptide—automated synthesis, degradation □ Amino acid sequence, computer controlled—polypeptide synthesis □ Degradation, polypeptide, Edman—automated □ Automated system—peptide synthesis, degradation

Classical methods have been employed in the chemical synthesis (1–6) and Edman degradation (7–10) of polypeptides and proteins. Recently, a number of laboratories have automated solid-phase polypeptide synthesis (11–14); in one instance (15), the automation of classical sequential analysis of polypeptides has been reported.

Studies in this laboratory have been directed toward the development of automated techniques that might be applicable to solid-phase synthesis and solid-phase degradation of polypeptides. Toward this goal an Automatic Chemical Reaction System (ACRS) (16) has been designed and constructed to carry out the physical operations involved in the synthesis and/or degradation following commands punched in a paper tape. Another phase of this research program has involved the development of computer approaches for generating the paper tape containing the commands for executing the synthesis or degradation (*e.g.*, amino acid additions, filtration steps, stirring sequences, deblocking steps, wash cycles, and activation steps).

Although the synthesis of polypeptides on the solid phase has been extensively studied and discussed (17–21), reports on solid-phase degradation have been limited to studies employing a resin-bound isothiocyanate functional group (22, 23) and to studies with the peptide bound to the resin but using methylisothiocyanate (MTC) in the degradation (24, 25). Dijkstra *et al.* (25) suggested that MTC is preferable to the classical phenylisothiocyanate (PTC) due to its higher solubility in aqueous solvents. They also suggested that PTC interacts unfavorably with the resin under their reaction conditions. Since Edman and Begg (15) demonstrated nearly quantitative coupling of PTC with free amines, and since the nature of the solvent could have a considerable influence on the interactions occurring within a chemical system, a study of the degradation using PTC in nonaqueous solvent systems seemed worthwhile. Information concerning the use of PTC with solid-phase degradative systems in nonaqueous solvents has been limited (26).

In this article, the authors report the application of ACRS to the solid-phase synthesis and degradation of a model polypeptide, L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-alanine. This model peptide was chosen to study: (*a*) the use of PTC for the degradation of polypeptides in conjunction with a solid-phase (polystyrene) matrix; (*b*) the solid-phase degradation in a nonaqueous solvent that would be more compatible with both PTC and the resin; and (*c*) the feasibility of automating the synthesis and Edman degradation of polypeptides by applying a computer-oriented automated approach.

EXPERIMENTAL

Chemicals—All reagents were of analytical grade. L-Amino acids,[1] trifluoroacetic acid (TFA),[2] and chloromethylated polystyrene[3] were used. *tert*-Butyloxycarbonyl (*t*-BOC) amino acids were prepared by the method of Schwyzer *et al.* (27).

Preparation of *tert*-Butyloxycarbonyl Alanine (*t*-BOC ala.) Resin—The *t*-BOC ala. resin was prepared according to the method of Merrifield (17). Specifically, chloromethylated polystyrene (25 g., 0.7 meq./g.) was refluxed for 48 hr. with *t*-BOC ala. (9.88 g.) and triethylamine (Et_3N) (7.65 ml.) in absolute ethanol (150 ml.). The resin was filtered and washed three times with ethanol (50 ml. each) and three times with methanol (50 ml. each) and then was dried *in vacuo*. Amino acid analysis of an acid hydrolyzate of resin gave an alanine content of 0.523 meq./g.

Automatic Chemical Reaction System (ACRS)—ACRS (16) is an electromechanical device, which was designed and constructed to execute automatically the physical operations normally involved in solid-phase synthesis or degradation. Specifically, it is capable of measuring and dispensing appropriate chemicals into a reaction vessel, stirring chemicals, filtering, timing events, and, generally, controlling the synthetic or degradative processes. The information for every operation is punched onto a paper tape. The paper tape is fed into the ACRS unit, which translates the punched holes into physical operations such as turning on a stirrer or operating valve networks. Obviously, the ACRS is merely assisting by executing operations which would normally be carried out by the investigator.

Table I—Sequence of Chemical Additions for Coupling an Amino Acid

Chemical Additions	Operation	Time, min.	Total Time, min.
1. Chloroform	Wash (three times)	2.0 each	6
2. *t*-BOC leu	Amino acid addition	2.0 each	6
3. DCC	Activating agent	120.0	120
4. $CHCl_3$	Wash (three times)	2.0 each	6
5. Ethanol	Wash (three times)	2.0 each	6
6. $CHCl_3$	Wash (three times)	2.0 each	6
7. 50% TFA	Deblocking reagent	30.0	30
8. $CHCl_2$	Wash (three times)	2.0 each	6
9. 10% Et_3N	Neutralization	10.0	10
10. $CHCl_3$	Wash (three times)	2.0 each	6
		Total time/cycle =	202

The principal component of this type of reaction system is the decoder which translates the command tape. In essence, this approach divides the synthesis or degradation into two categories: (*a*) those features that are primarily physical in nature (*e.g.*, measuring and adding chemicals), and (*b*) those features that are primarily chemical in nature (*e.g.*, removal of protecting groups and coupling of amino acids). By assuming the burden of the first category, the ACRS permits the investigator to concentrate more on the chemical aspects of the synthesis or degradation.

Preparation of Control Tape with Computers—In the automated approach discussed in this report, the sequence of operations involved in the chemical synthesis or degradation is incorporated onto a paper tape. Methods for preparing the command tape quickly and accurately are extremely important. Manual methods could be used to prepare the control tape. However, such methods are very time-consuming and increasingly subject to error as the number of commands increase. These difficulties led to a search for a more rapid and reliable means for preparing control tapes. Since the linear progression of commands with time is analogous in form to a one-dimensional array, a computer appeared to be an obvious candidate for setting up a simple array of commands of any reasonable dimension (*e.g.*, 30,000 commands). Once the computer program for generating the command array is available, the computer provides a control tape with speed (60 commands/sec.) and with reliability for any number of commands.

By using this approach for the synthesis of polypeptides, it is only necessary to specify the amino acid sequence, and the computer generates a one-dimensional array of commands for the complete synthesis. To specify the desired amino acid sequence, a set of data cards is prepared with the name of each amino acid punched onto a separate card. The cards are then placed in the data deck in the order that the amino acids occur in the polypeptide chain. Thus, if the sequence gly.val.leu.phe is desired, four cards corresponding to gly, val, leu, and phe, respectively, are arranged in order and inserted at the end of the data section of the program. For a 100-amino acid protein, 100 cards in the appropriate sequence are used. The program and data are read into the computer, which generates all of the commands involved in the complete synthesis of the peptide. With a functional program,[4] an investigator (with little or no programming experience) could generate a control tape of any desired length within minutes by simply supplying the desired amino acid sequence.

Computers—Computer programs[4] were written in the FORTRAN IV language and processed by an IBM 360-44 computer and a PDP-7 computer (University of Rochester). In this process, an array of commands was generated by the IBM 360-44 and copied

[1] Nutritional Biochemical Corp.
[2] Eastman Chemical Corp.
[3] Bio-Rad Laboratories.

[4] A listing of the computer programs is available from the authors.

Table II—Amino Acids Incorporated into the Resin during Synthesis

Leucine Addition Cycles	Average mmoles of Amino Acid/g. of Resin: Alanine	Leucine	Ratio of Alanine to Leucine: Experimental	Theoretical
0	0.495 ± 0.025	0.00	1:0	1:0
1	0.273 ± 0.030	0.241 ± 0.038	1:0.88	1:1
2	0.243 ± 0.040	0.476 ± 0.080	1:1.96	1:2
3	0.281 ± 0.013	0.800 ± 0.045	1:2.85	1:3
4	0.207[a]	0.840[a]	1:4.06[a]	1:4
5	0.195 ± 0.008	0.945 ± 0.010	1:4.86	1:5

[a] Values present are from one experiment since sampling from the second experiment was taken 5 min. after the addition of the fifth leucine and activating agent. (See *Discussion.*)

onto a magnetic tape. The magnetic tape was then read by the PDP-7 computer which produced a paper tape paralleling the commands on the magnetic tape.

Chemical Synthesis—The automated chemical synthesis of the model polypeptide, L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-alanine, was carried out using the sequence of operations shown in Table I. Initially, stock solutions of the various reagents were prepared and placed in the ACRS unit. These solutions consisted of: (*a*) 0.2 *M* *t*-BOC leu in chloroform ($CHCl_3$); (*b*) 0.2 *M* dicyclohexylcarbodiimide (DCC) in $CHCl_3$; (*c*) 10% Et_3N in $CHCl_3$; (*d*) 50% TFA in $CHCl_3$; (*e*) ethanol; and (*f*) $CHCl_3$. The desired amino acid sequence was read into the computer (*i.e.*, five data cards calling for leucine) along with the FORTRAN IV program deck. A one-dimensional array of commands for the synthesis of the peptide was then set up by the computer and copied onto a magnetic tape. The magnetic tape was then read by a PDP-7 computer which produced the control paper tape. The ACRS unit (through its decoder) executed the following operations from the tape.

A sample of deblocked *t*-BOC ala. resin (3.0 g.) in the reaction vessel was treated three times with $CHCl_3$ to wash and swell the resin. A solution of *tert*-butyloxycarbonyl-L-leucine (30 ml.) was then added, the mixture was stirred for 1 min., and a solution of DCC in $CHCl_3$ (30 ml.) was added. The resulting mixture was stirred at room temperature for 2 hr. and filtered. The resin was washed three times with each of the wash reagents: $CHCl_3$, ethanol, and $CHCl_3$, respectively (60 ml. of each reagent); the deblocking reagent [50% TFA in $CHCl_3$ (60 ml.)] was added. The deblocking mixture was stirred for 30 min. and filtered. Control experiments indicated that complete cleavage of the BOC group was obtained within 15 min. under these conditions. The resin was washed with $CHCl_3$ (60 ml.) three times, and 10% Et_3N in $CHCl_3$ (60 ml.) was added. The mixture was stirred for 10 min. and was filtered and washed three times with $CHCl_3$.

The resin was then ready for the next amino acid addition in subsequent positions of the control tape. The internal timer of the ACRS unit was set to move the control tape to the next position at 1.5-min. intervals. Following the last wash (Step 10 in Table I) in each coupling cycle, a sample of the resin was removed from the reaction vessel and dried. This resin (10–15 mg.) was then transferred to a hydrolysis tube, and a solution of 6 *N* HCl in 50% dioxane (1 ml.) was added. The tube was then sealed and incubated at 110° for 48 hr. Table II shows the average amino acid analysis of an acid hydrolyzate after each amino acid addition cycle for two experiments. In Fig. 1, the leucine residues on the resin during each experiment were plotted as a function of the reaction cycle (dotted line), together with the theoretically expected values (solid line).

Edman Degradation of the Model Peptide—As in the automated synthesis described, a control tape was prepared for the series of operations involved in the degradation of the resin-bound polypeptide. A computer program was used to set up an array of commands, which were copied onto a magnetic tape and subsequently translated into punched holes on a paper tape by the PDP-7 computer. The general sequence of operation is shown in Table III. In this case, stock solutions consisted of: (*a*) PTC–Et_3N–50% pyridine in dimethyl formamide (DMF) (1:1:8); (*b*) 50% pyridine in DMF; (*c*) $CHCl_3$; (*d*) 10% Et_3N in $CHCl_3$; and (*e*) 50% TFA in $CHCl_3$. The stock solutions were placed in the ACRS unit, and the paper control tape for the degradation was fed into the ACRS. Initially, the resin with the synthetic peptide attached was washed three times with 50% pyridine in DMF (60 ml.). The PTC solution (60 ml.) was then added. The reaction mixture was stirred under nitrogen for 120 min. and was filtered. After washing the resin three times with 50% pyridine in DMF and three times with $CHCl_3$, a solution of 50% TFA in $CHCl_3$ (60 ml.) was added. The resulting mixture was stirred for 120 min. and was filtered and washed with $CHCl_3$ (60 ml.) three times. The resin was stirred for 10 min. with 10% Et_3N in $CHCl_3$ (60 ml.) and was filtered and washed with $CHCl_3$ (60 ml.) three times.

Table III—Sequence of Chemical Addition for Edman Degradations

Chemical Additions	Operation	Time, min.	Total Time, min.
1. 50% pyridine in DMF	Wash (three times)	2.0 each	6
2. PTC	Derivative formation	120.0	120
3. 50% pyridine in DMF	Wash (three times)	2.0 each	6
4. $CHCl_3$	Wash (three times)	2.0 each	6
5. TFA in $CHCl_3$	Cyclization	120.0	120
6. $CHCl_3$	Wash (three times)	2.0 each	6
7. 10% Et_3N in $CHCl_3$	Neutralization	10.0	10
8. $CHCl_3$	Wash (three times)	2.0 each	6
		Total time/cycle =	280

This sequence of operations was repeated for each cycle (amino acid removal) in the degradation. The progress of the degradative process was monitored by analyzing the residual peptide attached to the resin after the final wash in each degradative cycle (Step 8 in Table III) as described. The change in leucine to alanine ratio indicated the progress of the degradation. Table IV shows the average amino acid analysis of acid hydrolyzates after each degradation cycle for two experiments. In Fig. 2, the leucine residues on the resin during each experiment were plotted as a function of the degradation cycle (dotted lines), together with the theoretically expected values (solid line).

RESULTS AND DISCUSSION

The studies with the model polypeptide, L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-alanine, have demonstrated the feasibility of applying computer-oriented automated techniques to the chemical synthesis and/or degradation of polypeptides and proteins. The ACRS unit with the computer-generated control tape carried out

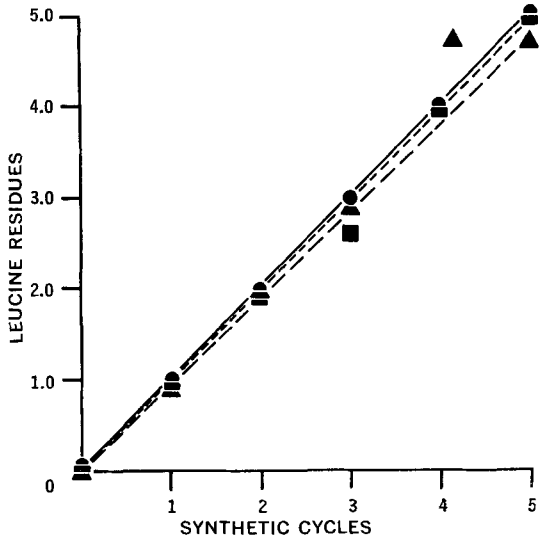

Figure 1—*The number of leucine residues present in an acid hydrolyzate of the resin is plotted as a function of the synthetic cycle. Theoretical values are indicated by the solid lines and circles. Experimental values are illustrated with dotted lines and with triangles in one case and with squares in the other. Leucine residues were determined with respect to the alanine content of the resin (Table II).*

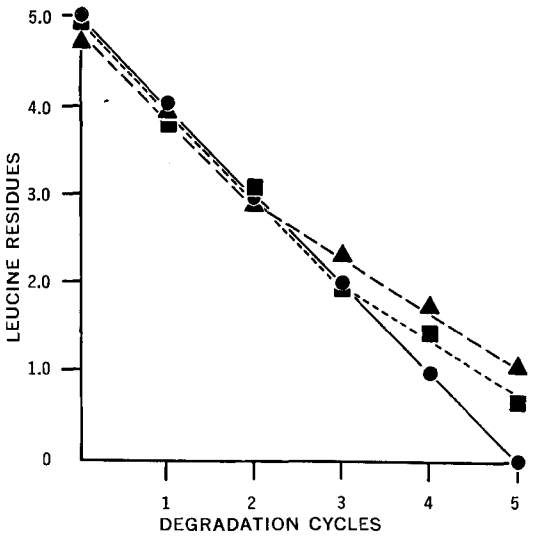

Figure 2—*The number of leucine residues present in an acid hydrolyzate of the resin is plotted as a function of the degradative cycle. Theoretical values are indicated by the solid line and circles. Experimental values are illustrated with dotted lines and with triangles in one case and with squares in the other. Leucine residues were determined with respect to the alanine content of the resin (Table IV).*

most of the physical operations involved in the synthesis and degradation of the model peptide. Progress of the amino acid incorporation into the growing chain was evidenced by the increase in leucine ratio in the amino acid analysis of an acid hydrolyzate of the resin-bound peptide.

Figure 1 compares the experimental values (dotted lines) for two different experiments with the theoretically expected values (solid line). The results indicate that the amount of leucine incorporated into the growing polypeptide chain is close to the theoretically expected amounts. Ultimately, it is desirable and necessary to drive the synthesis at each step to completion (at least 98% or higher yield at each step) to avoid a heterogeneous final product and a massive isolation and purification problem. For a synthesis to run under optimum conditions, information concerning the progress of each reaction step would be extremely important. Not only would such information provide an indication of the current status of the synthesis, it would also suggest future changes in the experimental approach which would accelerate the synthesis or optimize the yield of the reactions involved.

In Fig. 1 the triangle at Cycle 4 provides an example of the information that could be used to reevaluate the experimental approach. In this case, a sample of resin for acid hydrolysis was removed from the reaction vessel 5 min. after the addition of the new (activated) amino acid. The amino acid analysis of the resin demonstrated that leucine had been significantly incorporated into the polypeptide during this brief period. These results are consistent with those of Esko *et al.* (28) for the reaction of leucine with polypeptides on the solid phase. Other amino acids gave differences in reaction time for 90% completion extending from a few minutes to 24 hr. The rapid incorporation observed in this study would suggest a modification in reaction time in future studies and would result in the addition of more leucine residues per day. Reaction conditions [*e.g.*, Esko *et al.* (28)] that are specific for each amino acid could be readily incorporated into the computer program and the resulting paper command tape. In this case, when the amino acid sequence is specified, those conditions that are specific for a given amino acid (*e.g.*, reaction time, amount and concentration of chemicals to be added, and temperature of the reaction) would be generated by the computer in essentially the same time taken to generate the tape for reaction cycles that are identical for each amino acid. Information concerning the different chemical characteristics should be forthcoming in future studies and should permit the implementation of reaction conditions tailored to each amino acid.

The Edman degradation is an obvious choice for monitoring the current status of a synthetic operation at each major step since the free *N*-terminal amino acid is readily removed. The resulting peptide

Table IV—Amino Acids on the Resin following Edman Degradation

Degradation Cycles	Average mmoles of Amino Acid/g. of Resin		Ratio of Alanine to Leucine	
	Alanine	Leucine	Experimental	Theoretical
0	0.195 ± 0.008	0.945 ± 0.010	1:4.86	1:5
1	0.154 ± 0.012	0.600 ± 0.067	1:3.90	1:4
2	0.214 ± 0.002	0.623 ± 0.001	1:3.00	1:3
3	0.228 ± 0.008	0.483 ± 0.042	1:2.12	1:2
4	0.231 ± 0.012	0.322 ± 0.043	1:1.45	1:1
5	0.231 ± 0.037	0.212 ± 0.079	1:0.92	1:0

can then be analyzed as in this study, or the amino acid thiohydantoin derivative could be characterized and quantitated. A degradation following a coupling sequence could indicate the presence of free (uncoupled) *N*-terminal amino acid, while a degradation following a deblocking cycle would indicate the extent to which the preceding coupling cycle has gone to completion and the extent that the preceding amine protective group has been deblocked. The model peptide in this study does not lend itself to this type of interpretation because of the repeating sequence of leucine residues. In future studies, degradative methods will be used to evaluate the synthesis at each step for peptides containing different amino acids in adjacent positions.

This model peptide did indicate that the solid-phase degradation of polypeptides following computer-generated programs is indeed feasible in the nonaqueous solvent systems employed in this study. Figure 2 demonstrates the progress during the degradation of the model peptide in two different experiments (dotted lines). Beginning with the intact peptide attached to the resin, subsequent degradation steps reduced the leucine to alanine ratio by about one residue until the *N*-terminal of the residual peptide was within three amino acid residues of the resin. At this point, removal of additional leucine residues became more difficult. The nature of the decrease in cleavage is unclear and will be studied further. This lower cleavage rate has been reproduced in a number of experiments and is also in agreement with the results of Laursen (24). Attempts were made to overcome the reduction by employing different chemical preparations, solvents, and reaction conditions, but results consistent with Fig. 2 were obtained in each case. It is possible that the free amine is rendered inaccessible due to interactions between the smaller peptides and the resin. More experiments are needed to clarify this point.

Automation of the classical Edman degradation approach (15) has greatly accelerated the process of sequential analysis of proteins with the removal of 15 amino acid residues in 24 hr. The computer-oriented automated approach to solid-phase degradations discussed in this report should be equally useful in determining the amino acid sequence of proteins, with some modifications in the described method. Studies on the automated solid-phase degradation of naturally occurring polypeptides are currently being carried out in this laboratory

REFERENCES

(1) J. Rudinger, *Pure Appl. Chem.*, **7**, 385(1963).

(2) K. Hofmann and P. G. Katsoyannis, in "The Proteins," H. Neurath, Ed., Academic, New York, N. Y., 1963, p. 53.

(3) E. Schröder and K. Lubke, "The Peptides," vol. I, translated by E. Gross, Academic, New York, N. Y., 1965.

(4) H. D. Law, *Ann. Rep. Progr. Chem.*, **63**, 517(1966).

(5) M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Interscience, New York, N. Y., 1966.

(6) P. G. Katsoyannis and J. Ginos, *Ann. Rev. Biochem.*, **38**, 89(1969).

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

(8) *Ibid.*, **10**, 761(1956).

(9) D. Ilse and P. Edman, *Aust. J. Chem.*, **16**, 411(1963).

(10) W. R. Gray, *Methods Enzymol.*, **11**, 469(1967).

(11) R. B. Merrifield, J. M. Stewart, and N. Jernberg, *Anal. Chem.*, **38**, 1905(1966).

(12) K. Brunfeldt, J. Halstom, and P. Roepstorff, in "Peptides," E. Bricas, Ed., Wiley, New York, N. Y., 1968, p. 197.

(13) A. Loffet and J. Close, *ibid.*, p. 189.

(14) G. W. H. A. Mansveld, H. Hindriks, and H. C. Beyerman, *ibid.*, p. 195.
(15) P. Edman and G. Begg, *Eur. J. Biochem.*, **1**, 80(1967).
(16) A. M. Tometsko, patent pending (1969).
(17) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149(1963).
(18) *Ibid.*, **91**, 501(1969).
(19) R. B. Merrifield, *Science*, **150**, 178(1965).
(20) R. B. Merrifield, *Advan. Enzymol.*, **32**, 221(1969).
(21) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," Freeman, San Francisco, Calif., 1969.
(22) G. R. Stark, *Fed. Proc.*, **24**, 225(1965).
(23) L. M. Dowling and G. R. Stark, *Biochemistry*, **8**, 4728 (1969).
(24) R. A. Laursen, *J. Amer. Chem. Soc.*, **88**, 5344(1966).
(25) A. Dijkstra, H. Billiet, A. H. Van Coninck, and H. Van-Velthuyzen, *Rec. Trav. Chim.*, **86**, 65(1967).
(26) A. Schellenberger, H. Jeschkeit, R. Henkel, and H. Lehmann, *Z. Chem.*, **7**, 191(1967).
(27) R. Schwyzer, P. Sieber, and H. Kappeler, *Helv. Chim. Acta*, **42**, 2622(1959).
(28) K. Esko, S. Karlsson, and J. Porath, *Acta Chem. Scand.*, **22**, 3342(1968).

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NOTES

Effect of Mescaline HCl on Resistance of Male Mice to Histamine Stress

A. STANLEY WELTMAN, ARTHUR M. SACKLER, and LEROY JOHNSON

Abstract □ Single intraperitoneal injections of mescaline HCl caused significant decreases in the ability of albino mice to tolerate histamine phosphate when administered intraperitoneally 40 min. after mescaline. The dose levels of mescaline HCl ranged from 5–100 mg./kg. of body weight. Eight subcutaneous injections of mescaline (100 mg./kg. of body weight) administered during a 2-week period showed no difference between the LD_{50} values of the test *versus* saline control groups when challenged with histamine 24 hr. after the eighth and last dose of mescaline. No effects were noted in the body weights and growth patterns of the test mice in the 2-week investigation.

Keyphrases □ Mescaline HCl—effect on histamine tolerance, mice □ Histamine tolerance, mice—effect of mescaline HCl □ Hallucinogens, mescaline—acute *versus* prolonged effects on growth, histamine stress resistance

Previous investigations have demonstrated that LSD-25 stimulates adrenocortical activity and inhibits growth, metabolism, and gonadal and thyroidal function in female (1–3) and male rats (4). LD_{50} analyses of male rats given eight sequential injections of LSD-25 spaced during a 2-week period presented suggestive evidence of increased ability of the treated animals to tolerate histamine stress (5). Parallel studies with male mice receiving mescaline HCl (6) revealed identical, but somewhat smaller alterations in adrenal and thyroidal function but no effects on body weight and food consumption. Adrenal weights and adrenocortical activity [*i.e.*, thymus involution and white blood cell count (WBC) decreases] were significantly increased (6). The lesser effects produced by mescaline *versus* LSD-25 may be attributed to the lower potency of mescaline. The equivalent hallucinogenic dose of mescaline in humans is 4000 times greater than that of LSD (7). As with LSD-25 (3, 4, 8), various physiological findings have demonstrated the development of tolerance or accommodation of the treated mice to mescaline (6). The present investigation sought to determine acute *versus* prolonged effects of mescaline administration on body growth and the resistance of male mice to histamine stress.

EXPERIMENTAL

Three series of young male experimental albino mice (Carworth Farms, CFW) averaging 22 g. in body weight were selected for histamine LD_{50} analyses. Series I and II test and control mice were challenged intraperitoneally with histamine phosphate 40 min. after receiving a single intraperitoneal injection of mescaline HCl dissolved in normal saline or equivalent doses of saline. Series III mice received histamine phosphate 24 hr. after the eighth and final subcutaneous injection of mescaline HCl or normal saline. The eight injections were administered over a 2-week period on alternate days with the exception that on the 13th and 14th days, the two doses were given consecutively.

Series I consisted of 225 mice divided equally into three groups (two test groups and one saline control). The two doses of mescaline administered were 5 and 20 mg./kg. body weight. Series II consisted of an equivalent number of mice also divided equally into two test and one control groups. The test mice received a single dose of either 50 or 100 mg./kg. body weight of mescaline HCl. Series III was represented by a single test (100 mg./kg.) and normal saline group. In general, the various test groups were challenged with 5–6 dose levels of histamine phosphate and utilized 15–19 mice per dose level. The appropriate challenging doses of histamine, depending on the susceptibility of the test and control groups, ranged from 350–1450 mg. histamine base/kg. body weight. Finney's (9) method of probit analysis was used to calculate the LD_{50} values and dose–response lines.

To determine further the effects of various doses of mescaline on survival periods, the time of death following histamine phosphate