Table IV—Comparison of Different Dissolution Methods[a]

Method[b]	10A	10B	10C	10D'	5A	5B	5C	5D	Average
DT	3.0	2.9	3.3	8.7	3.2	3.1	1.5	2.2	3.5
3B	2.9	3.6	1.5	4.1	2.0	2.1	1.1	1.7	2.4
RB	2.8	2.0	1.5	3.7	1.2	1.2	1.0	2.5	2.0
FCL	1.0	1.0	1.0	1.0	1.0	1.0	1.2	1.0	1.0

[a] 10A = 10 mg. formulation of A, *etc.* [b] See footnote to Table III.

Comparison of 25- and 13-mm. Dissolution Chambers—Experience gained in these studies indicates that the 25-mm. chamber is the most useful for routine determinations. It accommodates all tablet sizes, it is easier to handle and use, and its filter seldom clogs during normal operation. Even at low flow rates (12–14 ml./min.), homogeneity of the system appears to be excellent. The 13-mm. chamber is preferred for precise work, especially that involving nondisintegrating material. It is especially useful in work with sublingual tablets.

Quantitative comparisons between the 13- and 25-mm. chambers can be made using Eq. 11 from Langenbucher's report (4):

$$T = k(Q_A)^{-0.2-0.5} \times (D_{p,o})^{1.5-1.8} \qquad \text{(Eq. 1)}$$

where T is the dissolution time (minutes), k is a constant, Q_A is the liquid velocity (centimeters per minute), and $D_{p,o}$ is the initial equivalent spherical particle diameter (centimeters). Since the liquid velocity equals the flow rate divided by the cross-sectional area of the chamber, it can be calculated that, using identical samples, the dissolution rates using the 13-mm. cell should be 1.4–2.3 times faster than those using the 25-mm. unit. For the 5- and 10-mg. experimental forumlations, the ratios of the semilog slopes (13 mm./25 mm.) are 1.7, 1.8, 2.0, and 2.3—in excellent agreement with the "theroretical" 1.4–2.3 range.

In conclusion, the results of these studies indicate that the modified dissolution chamber, consisting of commercially available units, is a useful tool in dissolution work. Studies on isosorbide dinitrate formulations, including commercial products, indicate that some differences in dissolution behavior exist between formulations, differences that may or may not be significant from an *in vivo* standpoint.

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NOTES

Immunochemical Studies on Linear Antigenic Polypeptides with a Known Repeating Sequence of Amino Acids

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Abstract □ The specificity of antibody to poly(L-tyrosyl-L-glutamyl-L-valylglycyl)glycine-1-^{14}C ethyl ester (I) was studied using polypeptides in which the valyl and tyrosyl residues were modified to an alanyl residue and either a phenylalanyl or a OMe-tyrosyl residue, respectively. All of these modified polypeptides cross-reacted with antibodies to I, indicating that they all have a similar conformation. It was concluded from the diminution of binding abilities of these modified polypeptides that antibodies to I show a greater specificity for the valyl and phenolic hydroxyl group of the tyrosyl residue of the homologous antigen.

Keyphrases □ Polypeptides, linear, antigenic—immunochemical properties □ Antibodies, polypeptide produced—specificity

The specificity of the antibody formed in response to an antigen is dependent on both the antigenic determinants present in the antigen and the genetic capacity of the animal to differentiate between, and respond to, those determinants. Neither the mechanism of specific antigen recognition nor the nature of its genetic control is well understood. With respect to antibody specificity, we have been studying the antigen poly(L-tyrosyl-L-glutamyl-L-valylglycyl)glycine-1-^{14}C ethyl ester (I) (1, 2). This article reports some cross-reaction and absorp-

Table I—Amounts of Protein Nitrogen Precipitated by Homologous and Heterologous Polypeptides

Polypeptide	Micrograms of Protein Nitrogen Precipitated at Equivalance Point[a]	Micrograms of Protein Nitrogen Precipitated by I after Absorption[a]	Percent of Protein Nitrogen Precipitated by Polypeptide[a]
(Tyr-Glu-Val-Gly)$_n$Gly, I	143	0	100
(Phe-Glu-Val-Gly)$_n$Gly, II	105	38	73
(Tyr-Glu-Ala-Gly)$_n$Gly, III	100	44	70
(OMe-Tyr-Glu-Ala-Gly)$_n$Gly, IV	95	46	67
(Phe-Glu-Ala-Gly)$_n$Gly, V	90	50	63

[a] Per milliliter of anti-poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C ethyl ester serum.

tion studies directed toward the characterization of the specificity of antiserum produced by rabbits against the antigen I. The following polymers were used: poly(Phe-Glu-Val-Gly)Gly methyl ester (II), poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C ethyl ester (III) (3–5), poly(OMe-Tyr-Glu-Ala-Gly)Gly-1-^{14}C ethyl ester (IV) (6), and poly-(Phe-Glu-Ala-Gly)Gly-1-^{14}C ethyl ester (V) (7).

RESULTS AND DISCUSSION

Four rabbits were immunized against poly(Tyr-Glu-Val-Gly)-Gly-1-^{14}C ethyl ester (I) using the previously reported protocol (5). The animals were bled for 25 days after the last injection of the antigen I. Each serum gave a positive precipitin reaction with the homologous polymer I. The serum from each animal was pooled, it being assumed that each animal had responded to the same antigenic determinants in this time interval (8).

In separate experiments, incremental amounts of each polypeptide (I, II, III, IV, and V) were added to 1-ml. aliquots of this pooled antiserum. All of the polypeptides cross-reacted with this serum. However, by quantitation of the precipitates by analysis for nitrogen (Kjeldahl), it was found that none of the heterologous polypeptides precipitated as much antibody as the homologous polypeptide I. These results are shown in Table I.

To quantitate the amount of antibody not precipitated by these heterologous polypeptides, a separate series of experiments was performed. Quantities equal to the equivalence-point amounts of each heterologous polypeptide were reacted with the pooled serum. After removal of the precipitate, 30 mcg. of the homologous polypeptide I was added to the resulting supernatant liquids; further precipitation was observed in each case and was quantitated by analysis for nitrogen.

The results suggest that all of the heterologous polypeptides (II, III, IV, and V) have the same conformation as the antigen I, since all of these polymers cross-react with anti-I-serum. From these results, it was assumed that the determinants of these heterologous polypeptides are in the same orientation as those of the antigen I. Thus, any observed differences in the abilities of the heterologous polypeptides (II, III, IV, and V) to bind with the antibody to I would be due only to the modification of the valyl and tyrosyl residues. By using this rationale, it is suggested that the antibody-combining sites show a diminution of bonding ability when the tyrosyl residue of the homologous polymer I is replaced by a phenylalanyl moiety, *e.g.*, polymer II. This may be due either to the inability of the phenylalanyl residue to fit the combining sites of the antibody molecule as well as the tyrosyl residue or to the presence, within the heterogeneous population of antibodies, of a population that shows a specificity for the phenolic hydroxyl group of the tyrosyl residue. The heterologous polymer, poly(Tyr-Glu-Ala-Gly)Gly (III), also shows a diminished ability to bind to anti-I-serum. It is suggested that the alanyl residue does not fit the antibody-combining sites as well as the valyl residue. Comparison of polymers II and V tends to confirm this suggestion, since the valyl polymer II precipitates more antibodies than the alanyl polymer V. Modification of two of the amino acid residues of the antigen as with polymers IV and V further reduces the precipitating ability. However, both of these modifications still cause over 60% precipitation of the available antibody. It is suggested that these results indicate that the antibodies produced by I are not very specific, provided that the heterologous polymers possess the same conformation as the homologous antigen.

EXPERIMENTAL

Immunochemical Procedures—Four rabbits were treated with poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C ethyl ester (I) at weekly intervals, using the immunization schedule previously described (5); 25 days after the last injection, all rabbits were bled using the standard heart puncture technique. Serum from each rabbit was tested for a precipitin reaction with the homologous antigen I, and a positive precipitin reaction was found in each case. The serum from each animal was pooled and this combined serum was used for the following experiments. It was assumed that the antibody produced by each rabbit after the same time interval was directed against the same antigenic determinants of poly(Tyr-Glu-Val-Gyl)Gly-1-^{14}C ethyl ester (I).

Quantitative Precipitin Reactions—To 1-ml. aliquots of the pooled rabbit serum was added incremental amounts of the polypeptide I. Each tube was made up to a total of 2 ml. with buffer (0.1 *M* NaCl–0.05 *M* $NaHCO_3$), incubated for 1 hr. at 37°, and then kept at 4° for 48 hr. The tubes were centrifuged in the cold, and the precipitates were washed twice with 1 ml. of buffer (0.05 *M* K_2HPO_4–1 *N* NaOH), pH 7.0. The total amount of protein precipitated was estimated by analysis for nitrogen (Kjeldahl). For each polypeptide (II, III, IV, and V), quantitative precipitin reactions were performed using the pooled rabbit serum, which was identical to and run simultaneously with that used for the polypeptide I.

Absorption Studies—The pooled rabbit serum was reacted with quantities equal to the equivalence-point amount of the heterologous polypeptides (II, III, IV, and V) as described previously. The corresponding precipitates were centrifuged out, and the supernatant liquors were poured off into separate tubes. To each supernatant liquor was added 30 mcg. of the homologous antigen, poly(Tyr-Glu-Val-Gly)Gly-1-^{14}C ethyl ester (I). The tubes were incubated at 37° for 1 hr. and then stood at 4° for 48 hr. The precipitates were centrifuged, collected, washed, and quantitated by analysis for nitrogen. Controls in which the serum was first absorbed with the homologous antigen I ascertained that the homologous antigen precipitated all of the antibody, since the supernatant liquor gave no further precipitation reaction when 30 mcg. of I was added.

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