Antigenicity of Polymers of Glutamyl Peptide in Humans. (24345)

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Antigenicity of glutamyl polypeptides has been the subject of many studies. Early interest arose from the fact that these peptides were one of the components of the capsule of *Anthrax Bacillus* and of *Subtilis-Mesentericus* group of organisms(1,2). Another more recent source of interest in antigenicity of these glutamyl peptides is the fact that polymers of them have been shown to have promise as plasma volume expanders(3). Interest has centered both on polymerized glutamyl peptides synthesized from straight chain peptide obtained from culture filtrates of *B. mesentericus* and on synthetic glutamyl peptides. The antigenicity of synthetic peptides has been studied by Stahman(4) and by Maurer (5) with somewhat discrepant results. This report presents data on antigenicity in humans of a branched chain glutamyl polymer which by actual test had molecular dimensions required for a satisfactory plasma volume expander in humans. As the question of antigenicity in humans of an otherwise suitable expander is of critical significance the findings are of considerable interest but must be confirmed by further studies.

Materials and methods. The glutamyl peptide polymer was prepared by previously described methods(3,6). The backbone and side chains were gamma linked peptides obtained from *B. mesentericus* culture filtrates. The molecular weight was approximately 80,000 by light scattering methods. The half life in blood stream of humans was approximately 15 hours. The material was essentially free of polysaccharide by the anthrone test, showing less than 2 μg of color-producing material/200 mg of peptide. It was dissolved as polysodium salt in isotonic saline to give a 2.5% solution. Immunization procedure in 24 human volunteers was essentially the same as that of Kabat and Berg(7) in testing antigenicity of dextran preparations. After preimmunization bleeding, subjects were skin tested on one forearm with intracutaneous injection of 50 μg of polymer, and with a saline control on the other forearm. Skin tests were read in 20 minutes, 24 hours and 72 hours. After the 72-hour reading 2.5 mg of polymer in saline solution was injected subcutaneously. Another similar strength immunizing dose was given 12 days later. Two weeks later the skin test was repeated and read at the same time intervals. Then blood was taken for serological studies. Those subjects showing any evidence of reaction were skin tested again one month later. *Quantitative precipitin tests* were performed by the method of Heidelberger and McPherson(8) using the Folin-Ciocalteu tyrosine reagent as described by Kabat(9). Sera were kept in refrigerator for 2 weeks to remove complement. Three ml aliquots of pre- and post-immunization sera were mixed in 15 ml conical bottom centrifuge tubes with varying amounts of polymer (Table II) and incubated at 37°C for 1 hour. They were then kept in refrigerator for 1 week with mixing twice a day. Any precipitate was collected by cold centrifugation and washed twice with cold saline prior to analysis. *Complement fixation* test was a standard test carried out overnight at 4°C. Pre- and post-immunization sera were tested against a range of concentrations of polymer.

Results. Positive findings in skin testing were limited to 6 subjects of the 24 tested (Table I). The other 18 subjects were negative and in none of the 24 was there any evidence of a pre-immunization reaction to the polymer.

In 3 subjects, No. 3, 4 and 16, on the first post-immunization skin test, there were erythematous areas, 2 to 4 cm in diameter, appearing within 20 minutes at site of injection. These disappeared within 2 hours. There was no itching accompanying these reactions. These 3 subjects showed negative reactions

TABLE I. Skin Tests.

Subject No.	1st immunization	2nd immunization	Post-immunization 20 min.	24 hr	72 hr	Subsequent retest 1 mo later
3	—	—	4.	—	—	No reaction
4	—	—	2.5	—	—	*Idem*
15	—	—	3.	—	—	1 cm diameter area of erythema with some itching in 20 min.; disappeared in ¾ hr
16	—	—	2.	—	—	No reaction
17	$R^{(1)}$	$R^{(2)}$	4.	—	—	2 cm diameter area of erythema lasting 8 hr; no wheal present
18	—	—	4.	2.5	—	No reaction

Numerical value is equivalent diameter area of skin reaction in cm.

$R^{(1)}$—Subject #17 showed 4 cm diameter area of erythema plus itching lasting for 2 hr at site of inj. on 4th day following 1st immunizing dose.

$R^{(2)}$—Subject #17 showed 4 cm diameter area of erythema at test site with 10 cm wheal occurring in 20 min. and lasting 10-12 hr.

when skin tested a month later. In subject No. 15, there was on post-immunization skin testing, a 3 cm erythema appearing within 20 minutes and disappearing within an hour. This subject also showed on skin testing one month later, a 1 cm area of erythema at site of injection, accompanied by some mild itching, appearing within 20 minutes and disappearing in 2 hours. Subject 18 had a 4 cm diameter area of erythema at site of injection on post-immunization skin test injection, persisting for 24 hours. This subject showed no reaction on skin testing one month later. None of these 5 subjects showed a wheal at any of the areas of erythema.

Only one subject had what was thought to be a significantly positive reaction. This subject, No. 17, showed a 4 cm diameter area of erythema plus itching, lasting 2 hours, at site of injection on fourth day after first immunizing injection. Prior to the second immunizing injection a skin test showed a similar reaction occurring at site of injection within 20 minutes and lasting 10 or 12 hours. A 1 cm wheal accompanied this reaction. The second immunizing dose was therefore omitted. This subject also showed a 4 cm diameter area of erythema at site of injection, accompanied by a wheal, and lasting 24 hours on the first post-immunization skin test as well as a 2 cm diameter erythematous area at site of injection with no wheal but lasting 8 hours, when skin tested one month later.

According to Kabat and Berg(7) only values of 2 μg or greater of precipitable antibody N/ml in a given serum, or a 2 μg or greater rise in precipitable antibody N/ml of serum between pre- and post-immunization sera, is considered significant. According to these criteria only 1 subject, No. 15, showed a positive reaction (Table II).

The results of the complement fixation tests were completely negative in that serum of none of the 24 subjects showed complement fixation with the polymer when used in concentrations varying from 10 to 500 μg with undiluted serum.

Discussion. The results show completely negative reaction to all 3 tests in 18 of the 24 subjects. Of the remaining 6, 4 show what was considered a questionable or minimal skin reaction 2 weeks after the last immunizing dose, a negative reaction one month later and negative serological tests. It is believed that at most there is questionable or minimal sensitivity in these 4. Subject 15 showed minimal skin reaction and the only positive precipitin in the group. Subject 17 showed what we consider the only real skin reaction but negative serological tests. It is pertinent that subject 17 was a known severely allergic person, highly sensitive to many common allergens.

It is interesting to compare these results to

TABLE II. Precipitin Tests.

	g of polymer added							
Subject #15	2.5	10	20	25	35	50	100	200
Pre-immunization	0	0	0	.4	0	0	0	0
Post- "	.3	3.7	5.6	0	0	0	0	0

Values refer to μg of antibody nitrogen precipitated/ml of serum.

those obtained by Kabat and Berg(7) in studying antigenicity of Dextran, another plasma expander which had been found clinically acceptable. As part of a larger study group Kabat tested 3 different samples of clinical dextran in 24 subjects. Of the 24, 9 showed positive skin tests after immunization with 7 of the 9 showing a 1+ or stronger reaction. A 1+ reaction was defined as a wheal of about 3-6 mm greater than that obtained in the same subject with the saline control with a surrounding erythema when read 15 to 20 minutes after the injection period.* Of these 9, 7 showed a rise of 2 μg or more of precipitable antibody nitrogen/ml of serum after immunization and 1 of the remaining 2 had more than 2 μg of precipitable antibody nitrogen/ml of serum prior to immunization. Maurer(10) obtained generally similar results in testing 4 samples of clinical dextran in 30 subjects, 8 of whom showed significant post-immunization antibody N rises with 6 of these 8 developing positive post-immunization skin tests.

It is difficult to make an exact comparison but insofar as the positive skin reactions in Kabat's study showed wheals as well as erythema plus other signs of reaction generally lasting 1 day or longer in the more severe cases (those showing a 2+ or 3+ reaction), as well as significantly positive serological tests, it seems that glutamyl peptide polymer compared favorably with dextran with respect to weakness of antigenicity in humans.

Many attempts to immunize rabbits and guinea pigs with our linear or polymerized glutamyl peptides have been negative.

Summary. 1) Antigenicity of a polymer of glutamyl peptide has been tested in 24 human volunteers. The polymer was of molecular weight and dimensions suitable for use as a plasma volume expander in humans. Skin tests, precipitin tests and complement fixation tests were performed on all subjects. 2) The results show 18 subjects with completely negative reactions by all 3 tests. There were 4 skin test reactions of dubious significance and one of real significance. There was one significantly positive serological reaction in a person showing a minimal skin reaction.

* Personal communication from Dr. Kabat.

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Effects of Reserpine on Cholesterol Levels in Cholesterol-Fed Rabbits.* (24346)

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It has been suspected that severe emotions and what is called "mental stress" might have some relation to elevations in blood cholesterol. Lyons(1) reported experiments in which serum cholesterol elevations were observed in cats when placed in cages surrounded by barking dogs. Selye(2) described a biphasic response of serum cholesterol in his

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