

Allergy to trypsin

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Trypsin (Parezyme) is used extensively in the treatment of a variety of disorders.¹⁻²⁶ These conditions include thrombophlebitis, peripheral vascular disease, venous thrombosis, postoperative edema, traumatic swelling, hematomas, ulcerative lesions, postphlebotic syndrome, etc. It is available commercially in two vials, one containing lyophilized trypsin, 12,500 units, N.F. (5 mg.), and the other containing 1 ml. of aqueous diluent. The diluent contains 5 per cent denatured gelatin and preservatives.

The present study was undertaken to determine the antigenicity of this product, and is divided into two parts. The first deals with the detection of precipitating antibodies in experimental animals sensitized to trypsin. The second reviews a number of clinical reports intended to reveal the frequency of untoward reactions to trypsin and the serologic changes in patients treated with this product.

METHODS

Animal experiments

Rabbits and guinea pigs were sensitized with trypsin according to several techniques.

Twelve New Zealand white rabbits of approximately 3 kilograms each were used. These were divided into four groups.

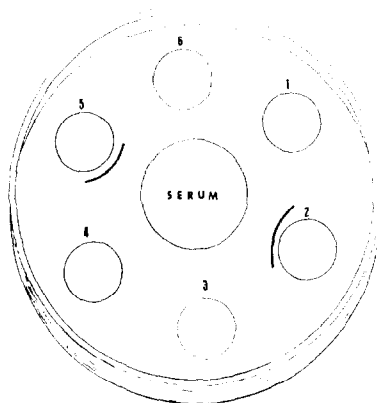
Group 1 (4 rabbits). Each rabbit received 1 ml. (5 mg.) of trypsin intramuscularly followed by 0.2 ml. intracutaneously for three doses.

Group 2 (3 rabbits). Each rabbit received 1 ml. (5 mg.) of trypsin intramuscularly followed one week later by 0.1 ml. intracutaneously in three separate skin sites in dilutions of 1 to 100, 1 to 50, and 1 to 10. The purpose of

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**Fig. 1**

Ouchterlony gel diffusion test showing precipitin bands of one rabbit serum (group 1) against trypsin (wells 2 and 5). Other wells are controls.

injecting these various dilutions was to determine at what point local inflammatory skin reactions occurred.

Group 3 (3 rabbits). Each animal received 0.2 ml. (1 mg.) trypsin subcutaneously every other day for three doses. One week after the third subcutaneous dose, each rabbit received 0.2 ml. (1 mg.) intramuscularly weekly for two doses.

Group 4 (2 rabbits). Each rabbit received 1 ml. gelatin alone according to the schedule indicated for group 3. This was the control group.

All animals in each group were bled and killed 1 week after the last injection. In addition to serologic tests, various tissues were studied for pathologic changes. The Ouchterlony technique was used to determine precipitating antibodies. After reconstitution each milliliter of antigen solution contained 5 mg. of trypsin. From this preparation, dilutions of 1 to 10, 1 to 100, and 1 to 1,000 were made with 0.85 per cent Merthiolated saline. Precipitin bands were demonstrable by this technique in the serum of one rabbit in group 1 at 1 to 10 dilution and in the sera of two animals in group 2 in all dilutions through 1 to 100, but not in 1 to 1,000 (Fig. 1). All the rest of the sera were negative. Biopsy specimens of skin at the point of intracutaneous injection of trypsin showed evidence of an acute inflammation, but no microscopic changes characteristic of an Arthus-type reaction. Several rabbits in groups 1, 2, and 3 showed focal pulmonary emphysema, but no other significant pathologic changes. Pathologic lesions in the kidneys and brain that may be found in the normal rabbit population were excluded.

The above experiment was repeated with ten guinea pigs. These were divided into two groups. Group 1 (6 animals) was sensitized with trypsin intramuscularly; each animal was injected with 1.0 mg. of trypsin daily for 7 consecutive days. Group 2 (4 guinea pigs) was the control. These animals were injected intramuscularly with 0.1 ml. of gelatin containing diluent alone daily for 7 days. Fifty-six days from the beginning of the sensitization program, all animals were challenged intravenously with 0.1 mg. of trypsin per 100 Gm. of body weight, receiving a total volume of 0.1 ml. per 100 Gm.

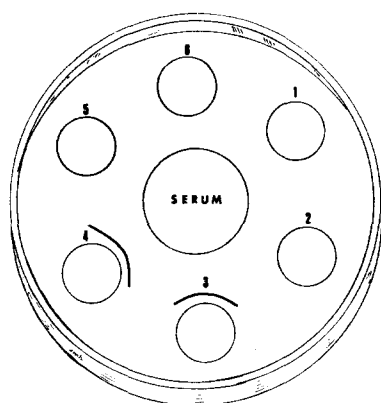


Fig. 2

Ouchterlony gel diffusion test showing precipitin bands of guinea pig serum (center well) against trypsin (wells 3 and 4).

Three of the sensitized guinea pigs (group 1) died in anaphylactic shock shortly after the challenging dose was administered intravenously. No such reaction occurred in the control animals.

The sera of two guinea pigs dead from anaphylactic shock showed positive bands to trypsin upon examination by the Ouchterlony technique (Fig. 2). The controls were negative.

RESULTS

Clinical studies

The following analysis of adverse reactions to trypsin is based on information obtained from the literature and from the manufacturer.

Fisher and Wilensky⁴ report only mild transient reactions and a negligible incidence of nonfatal embolic phenomena in 333 patients treated with trypsin intramuscularly and intravenously. Other than this reference, all the available reports of untoward reactions concern a related product, namely, chymotrypsin (Chymar). Chymotrypsin is a proteolytic enzyme of beef pancreas and is obtained by the action of trypsin on its precursor, chymotrypsinogen. Present available evidence discloses no information regarding the similarity of antigens in trypsin and chymotrypsin. Thus, MacLaren and Aladjem²⁷ present a case of nasal and conjunctival allergy brought about by the inhalation of chymotrypsin. Rose²⁸ reports an anaphylactic reaction to aqueous chymotrypsin in a previously nonsensitized patient. Wade²⁹ refers to one mild anaphylactoid reaction to the same product. Liebowitz and Ritter³⁰ cite one instance of anaphylactic shock to chymotrypsin after sensitization resulting from the previous administration of this product. Mozan³¹ found only three allergic reactions following the intramuscular injection of chymotrypsin in seventy-eight patients for 4,666 times. One reaction was severe but easily controlled anaphylactic shock. The other two cases were accompanied by nasal symptoms and urticaria. Luft³² also refers to possible anaphylactic reactions in patients treated with chymotrypsin. Sherline and Blackwell³³ discuss one instance of severe but reversible

anaphylactic reaction, and Gherini and Hovde³⁴ describe several reversible reactions in patients receiving chymotrypsin. Howell³⁵ interprets as anaphylactic a severe reaction involving the brain following the injection for the second time of chymotrypsin; one would suspect, however, that this may well have been caused by brain embolus. Ferrara and colleagues³⁶ report two additional reactions to this product accompanied by skin-sensitizing antibodies and passive transfer but negative precipitin tests. Howe and associates³⁷ report two instances of laboratory-acquired allergy to chymotrypsin and trypsin. One patient showed positive passive transfer of skin-sensitizing antibodies to chymotrypsin.

Accounts of adverse clinical reactions from trypsin (Parezyme) were obtained from the manufacturer's files. The total number of doses manufactured and distributed since 1953 is 10,124,000. Information obtained by the manufacturer from its field representatives and correspondence with physicians indicate a total of 178 adverse reactions to trypsin during this interval. Most of these reactions were of a minor nature and transient. Many of them, it would seem, were not actually related to trypsin administration. Therefore, the total incidence of all of these side effects, even including those which are questionable, is approximately one reaction per 58,000 doses.

Of this total of 178 adverse reactions, ninety-six presented nothing to suggest that they were of an allergic nature. In eighty-two instances, the manifestations were allergic. The nonallergic reactions consisted of such manifestations as local pain and swelling at the point of injection, fainting, dizziness, nausea, vomiting, abdominal pain, and about four cases of embolic phenomena. The allergic reactions consisted mostly of generalized urticaria and angioedema, rhinorrhea, sneezing, occasional asthma, and in two instances fatal anaphylactic shock. Of the eighty-two allergic reactions, forty-eight were in patients known to have been treated previously with trypsin, which indicates prior sensitization. In two instances, patients previously treated without any appreciable local reaction developed massive inflammation at the site of the subsequent injection. This suggested the possibility of Arthus-type reaction. As indicated above, there were two fatal anaphylactic reactions in this group. In each instance, the patient received trypsin previously without any reaction. In one case, trypsin was given following repair of episiotomy. The patient, a 21-year-old nurse, suddenly developed generalized purpura and itching, cyanosis, and shock, and died of circulatory collapse within 2 minutes. Autopsy showed diffuse purpura, recent hemorrhage into the atrioventricular node of the heart, focal necrobiosis of subnodal myocardium, ring hemorrhages in the hypothalamus, acute pulmonary congestion and emphysema, and partial obstruction of the tracheobronchial tree by hemorrhagic mucus. The second patient died in circulatory collapse and shock within 15 minutes after the administration of 0.5 c.c. of aqueous trypsin. However, it is not altogether certain whether this death was the result of anaphylactic shock or an embolus.

Clinical investigation

An attempt was made to determine the sensitization capacity of intramuscular trypsin in man. It was first necessary to establish the dilution of the

crystalline product which, when employed both by the scratch and by the intracutaneous technique, would not yield a nonspecific skin reaction but which would still give a positive skin test in clinically sensitive patients.

One hundred six patients were tested intracutaneously and eighty-five were tested by the scratch technique with trypsin. As a result of these tests, the conclusion was reached that a 1 to 200 dilution of aqueous trypsin (Parenzyme) is satisfactory for intracutaneous tests. Trypsin (Parenzyme) may be used for scratch testing when diluted with 50 per cent aqueous gelatin or with glycerine.

Ninety-four individuals were injected intramuscularly with 1 ml. (5 mg.) of trypsin. Of these, thirteen received but one injection; seventy-six received two injections 1 week apart; one, three weekly injections; and four, four weekly injections. Each patient was skin-tested with trypsin before beginning treatment and after the last injection. Only two patients out of ninety-four developed mild allergic reactions. One (No. 156) showed generalized urticaria 36 hours after the second injection. This patient had a negative skin test before and a positive skin test 2 days after the last treatment with trypsin. The second patient (No. 188) developed generalized urticaria and angioedema within a few hours after the second injection. This became more severe in 24 hours, but responded easily to treatment. This patient also had negative skin tests to trypsin prior to, and positive skin tests 1 week after, the last trypsin injection. Passive transfer test results were positive in the second instance. Both of these patients (Nos. 156 and 188) were atopic individuals.

Seven out of ninety-four patients receiving trypsin by injection showed a 2 plus skin reaction on intracutaneous testing prior to treatment. These patients may have received trypsin previously. None of these individuals developed systemic reactions following trypsin injections. Twelve patients who had a negative skin test before injection developed a 2 plus intracutaneous reaction after two injections of trypsin (Parenzyme) without showing any clinical manifestations. Passive transfer test results were positive in only one of these patients.

Serologic studies

In vitro tests were carried out with the serum of patients reacting to trypsin. Each milliliter after reconstitution contained 5 mg. of trypsin. From this preparation dilutions of 1 to 10, 1 to 100, and 1 to 1,000 were made with 0.85 per cent Merthiolated saline. The Ouchterlony gel diffusion technique was employed to determine the presence of precipitating antibodies. Twenty-five sera samples (controls) were obtained from untreated individuals. These sera showed no specific precipitin bands. Seventy-nine sera were obtained from forty patients who received at least two weekly injections of trypsin (Parenzyme). Of these, nineteen sera showed specific bands. Seventeen sera showed bands of precipitation which varied between multiple heavy lines and very thin lines. In some instances, these lines tended to disappear. Two sera, however, showed persistent bands indicating the presence of precipitating antibodies to trypsin. Neither of these two patients had positive skin tests or clinical reactions to trypsin. No precipitins could be demonstrated in the sera of the two patients (Nos. 156 and 188) who had positive skin tests. All the sera obtained after

trypsin injections were tested by the tanned cell hemagglutination technique as described by Stavitsky and Arquilla³⁸ and others.³⁹ Parenzyme used in the hemagglutination tests was diluted with 10 to 3 HCl so that each milliliter contained 25 mg. Then it was diluted with phosphate-buffered saline so that each milliliter contained 0.5 mg. and was adjusted to the appropriate pH. Thus far, hemagglutination tests have been entirely negative. Whether this is because of an absence of these antibodies even in the sera showing precipitins or the result of technical difficulties in working with this enzyme system remains to be determined.

DISCUSSION

Our experience indicates that anaphylactic sensitivity to trypsin develops more readily in guinea pigs than in rabbits. Rice and co-workers⁴⁰ sensitized guinea pigs with trypsin in oil at a dose of 5 mg. per kilogram twice daily for 7 days. The animals were challenged 60 days later by the injection of 1 mg. per kilogram of trypsin in saline into the heart. Sensitized guinea pigs were also challenged by the aerosol administration of 1 per cent trypsin. Ten guinea pigs showed evidence of nonfatal anaphylactic shock following the intramuscular injection of the challenging dose. Severe and fatal anaphylaxis occurred from exposure to trypsin by aerosol. Administration of trypsin by intracardiac injection caused fatal anaphylaxis in all sensitized animals. Challenging rabbits with the same technique failed to induce fatal anaphylaxis. Positive precipitin reaction occurred with concentrations of trypsin antigen as low as 0.05 per cent. Similar evidence of the antigenicity of trypsin in guinea pigs has been reported by Achalmé⁴¹ and by Broeck.⁴² Roekli and Campbell⁴³ have prepared rabbit antibodies against chymotrypsin by subcutaneous administration of the product in complete Freund's adjuvant. Silva²⁵ demonstrated that the intravenous injection of trypsin in fairly large doses produces anaphylactoid shock in animals—possibly because trypsin may cause the liberation of histamine. However, other factors may be responsible for this shock, because animals treated with antihistamines are not protected. Mirskey and Fried⁴⁴ reported that the intravenous administration of trypsin causes kidney and liver damage as well as intravascular clotting. This effect could well be responsible for systemic shock.

As might be expected, injections with trypsin may cause some adverse reactions which may be allergic or nonallergic. It appears that the diluent vehicle, namely, gelatin, is inactive antigenically. The incidence of allergic reactions from trypsin (Parenzyme) is quite low compared to other popular biologic products such as penicillin or horse serum-containing preparations. Skin-testing with trypsin is mandatory prior to treatment. The physician administering trypsin should be prepared to treat such patients promptly in case of an anaphylactic reaction by use of the usual measures, including administration of epinephrine, ephedrine, Levophed, oxygen, antihistamines, and corticosteroids.

SUMMARY AND CONCLUSIONS

Precipitating antibodies are found in rabbits and guinea pigs sensitized with trypsin. Anaphylaxis occurs readily in guinea pigs similarly sensitized. Sensitization with trypsin causes no pathologic changes in rabbits or guinea pigs.

An analysis of all reported reactions from trypsin (Parenzyme) injections indicates a relatively low incidence of allergic reactions.

Intracutaneous or scratch-testing with trypsin should precede its administration. Two out of ninety-four patients developed allergic reactions (urticaria) following multiple injections with trypsin. Both patients had positive skin tests; for one patient the passive transfer test was positive.

Precipitating antibodies against trypsin in treated individuals, as determined by the Ouchterlony technique, were mildly present in only two instances. No hemagglutinating antibodies were detectable.

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