

Inhalation sensitization to trypsin

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A patient is reported who developed asthma during an eight-year occupational exposure to crude trypsin and chymotrypsin powders. Precipitation of asthmatic symptoms by trypsin was confirmed by provocative inhalation tests. The patient also manifested immediate sensitivity reactions when skin tested to a trypsin solution. Both inhalation and skin tests were negative in control subjects. The implication of sensitivity developing to aerosolized trypsin is discussed in relation to the long-term use of this agent in some asthmatic patients.

Trypsin, obtained from bovine and porcine pancreas, has been used in the aerosol therapy of a number of bronchopulmonary conditions,^{1, 2} including asthma with or without infectious complications.^{3, 4}

We have recently studied a patient who was employed for eight years in the extraction of crude trypsin powder preparations from bovine and porcine pancreatic tissue. The patient wore no mask during this extraction procedure and, therefore, repeatedly inhaled the trypsin powder. He manifested upper and lower respiratory tract symptoms of increasing severity while at work. There was a considerable reduction in these respiratory symptoms on weekends when the patient was away from work. Both skin tests and provocative inhalation tests were carried out on the patient and on control subjects from our Allergy and Immunology Clinic population. Following the initial detailed study, the patient was re-evaluated after a one year period, during which he had avoided all contact with trypsin.

Case report

J. R., a 43-year-old white man, was admitted for the first time to the Hospital of the University of Pennsylvania on Feb. 14, 1964, because of cough, wheezing, and paroxysmal dyspnea. The patient had experienced no significant respiratory complaints until approximately 7 years prior to admission. At that time, he had been working for one year in a chemical company processing crude trypsin preparations from pancreas of bovine and

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Received for publication April 25, 1966.

porcine origin. He noted consistent precipitation of coughing and sneezing related to the handling of this crude trypsin powder. These symptoms increased progressively throughout the work week, with considerable improvement while at home over weekend periods. He also noted symptoms to a lesser extent when handling chymotrypsin powder. The acetone used as a solvent in the extraction of this trypsin preparation would also aggravate his symptoms to some degree. These symptoms persisted at work over the next 6 year period. In September, 1963, daily asthmatic attacks with a production of mucopurulent sputum occurred while the patient was at work. These episodes became progressively more severe until the time of his admission. He had been hospitalized elsewhere in March, 1963, for evaluation of his pulmonary complaints and for hypertension (blood pressure in the range of 175/120 mm. Hg). Chest x-rays taken at that time revealed a bleb in the right lung but no evidence suggestive of bronchiectasis.

It is of interest that, although the patient denied any symptoms suggestive of allergic respiratory disease prior to the contact with trypsin powder, he had noted that contact with cement dust and certain animal danders would also precipitate coughing and sneezing to a milder degree during the past 7 years. There was a past medical history of several episodes of pneumonia and an episode of rheumatic fever manifested by arthritis without carditis, both in childhood. The patient's family history was quite strong for allergic diseases, with allergic rhinitis and/or asthma in several members of his immediate family.

Physical examination at the time of admission showed a blood pressure of 170/110 in the right arm and 150/100 in the left arm. There was slight attenuation of the arterioles of the fundus. Fine expiratory wheezes were heard throughout all lung fields, with slight accentuation in the area over the middle lobe of the right lung. There were no bruits in the area over the kidneys.

Laboratory studies included a hemoglobin level of 16.6 Gm. and a white count of 7,200 with 2 per cent eosinophils. Studies which were within normal limits included two lupus erythematosus preps, a fasting blood sugar, blood urea nitrogen, serum sodium, potassium, chloride, and CO_2 , electrophoretic pattern of the serum proteins, electrocardiogram, catecholamines of a 24 hour urine specimen, and urine analysis. Several cultures of the sputum showed no pathogens. A smear of the sputum revealed many polymorphonuclear leukocytes with 15 per cent eosinophils. Chest x-ray showed a probable bleb in the lower field of the right lung. A retrograde aortogram showed nothing to suggest a renovascular cause for the hypertension. Extensive pulmonary function studies (Table I) revealed reductions in the vital capacity, timed vital capacity, maximum expiratory flow rate, maximum breathing capacity, and peak flow rate. These rates improved significantly after isoproterenol nebulization. Skin tests for reagin antibody to a wide variety of indoor and outdoor inhalant allergens* were carried out. There were significant positive reactions on scratch or intradermal testing to the danders of cat, dog, rabbit, and horse, as well as to pyrethrum (Table II). The tests utilizing extracts of beef and pork were negative by both scratch and intradermal techniques.

When the patient's symptoms failed to respond to the usual bronchodilator agents, he was treated with large doses of prednisone for one week, with prolonged symptomatic improvement. The prednisone dosage was gradually tapered from 30 mg. per day to complete absence of steroid therapy over a period of several weeks, with no recurrence of symptoms. It was felt that the patient also had mild to moderate essential hypertension, and he was treated with reserpine and thiazide agents. Over the succeeding year, the patient resigned his job, avoiding all contact with biological powders. He felt that this resulted in marked improvement in his respiratory symptoms while he was receiving no steroids or bronchodilator agents. He continued to note mild dyspnea when exposed to large concentrations of dust, but there were no frank asthmatic episodes and he was asymptomatic during moderate exertion. He had voluntarily discontinued taking his anti-hypertensive medication.

He was readmitted for re-evaluation of his respiratory status in January, 1965, almost

*Hollister-Stier Laboratories, Spokane, Wash.

Table I. *Baseline pulmonary function studies*

	<i>February, 1964</i>	<i>January, 1965</i>	<i>Predicted</i>
Vital capacity	2,900 ml.	3,300 ml.	4,400 ml.
Timed vital capacity			
1 second	60%	52%	83%
2 second	84%	69%	94%
3 second	92%	80%	97%
Maximum expiratory flow rate	102 L./minute	—	400 L./minute
Maximum midexpiratory flow rate	—	42 L./minute	130-395 L./minute
Maximum breathing capacity	32 L./minute/M. ²	32 L./minute/M. ²	64 L./minute/M. ²
CO ₂ tension (arterialized)	44 mm.	49 mm.	43-53 mm.

Table II. *Allergy skin testing*

<i>Positive tests</i>	<i>Negative tests</i>
Cat, dog, rabbit, horse, pyrethrum	Cattle, feather, kapok, karaya, trees, grasses,
Parenzyme, 1:200 (strong positive in	plantain, ragweed, Alternaria, Aspergillus,
February, 1964)	Helminthosporium, Hormodendron
Parenzyme, 1:200 (moderate positive in	
January, 1965)	
Trypsin, 100 mg.% (positive scratch)	

one year after complete avoidance of any contact with trypsin powder. At that time, physical examination demonstrated persistent slight increase in the anteroposterior diameter of the chest with slight prolongation of the expiratory phase. However, there was no wheezing. His blood pressure was still abnormal, in the range of 170/110, without any evidence of congestive heart failure. Repeated pulmonary function studies (Table I) showed residual decreases in the timed vital capacity and maximum expiratory flow rate, which did not return to normal after bronchodilator nebulization. The decrease in the maximum breathing capacity did return to normal after bronchodilators. There were slight nonspecific abnormalities of the electrocardiogram with inversion of T waves in V₅ and V₆, as compared with previous examinations in 1964.

MATERIALS AND METHODS

Skin testing

In addition to the skin testing noted above, the patient was tested intradermally with 0.02 ml. of a trypsin solution (Parenzyme, diluted 1:200). This preparation had been noted by Criepp and Bearn⁷ to elicit no significant incidence of false positive reactions. Similar trypsin intradermal skin tests were carried out in a control population of 50 allergic individuals without histories suggestive of trypsin sensitivity.

Provocative inhalation testing

After stable base-line measurements of the timed vital capacity were obtained, the patient received several aerosols through a Devilbiss No. 40 nebulizer without knowledge of the contents or the purpose of the nebulization. He first inhaled 1.0 ml. of a physiologic saline solution and waited 1½ minutes.

The vital capacity studies were then repeated. After the patient rested an additional 15 minutes, the timed vital capacity was again determined. He then inhaled 1.0 ml. of a 1 mg. per cent saline solution of the crude trypsin powder to which he was exposed at work. One and one-half minutes later, the timed vital capacity was again determined and the patient rested.

In similar fashion, the patient received successive inhalations of increasing concentrations of a crude trypsin preparation followed by similar pulmonary function testing. These studies were carried out during the hospital admissions of February, 1964, and January, 1965. Similar provocative inhalation tests with trypsin solutions were carried out in two control subjects with mild perennial asthma and with no history of exposure to trypsin in the past.

RESULTS

Skin testing

In February, 1964, the patient manifested a strong positive (i.e., 15 mm. wheal and 40 mm. erythema) reaction with pseudopods to a 1:200 dilution of Parenzyme (Table II). In January, 1965, there was a moderate reaction to this intradermal test with Parenzyme; a scratch test to a 100 mg. per cent aqueous solution of the trypsin preparation obtained from the patient's place of employment showed a strong positive (12 mm. wheal and 30 mm. erythema) reaction. Intradermal tests with the 1:200 dilution of Parenzyme in 50 allergic patients from the Allergy and Immunology Clinic used as controls showed only one similar positive reaction.

Provocative inhalation testing

In February, 1964, the provocative inhalation of saline and then trypsin solutions in concentrations up to and including 100 mg. per cent led to no symptoms nor any significant change in the vital capacity and maximum midexpiratory flow rate (Table III). However, when the patient had inhaled only about 0.3 ml. of the 1.0 Gm. per cent trypsin solution, he was forced to stop because of the onset of severe coughing and wheezing associated with a marked decrease in vital capacity and maximum midexpiratory flow rate. These symptoms and abnormal pulmonary function tests persisted for over one hour, despite the use of nebulized bronchodilators. The patient remained acutely symptomatic for over 24 hours, requiring parenteral bronchodilator therapy.

In January, 1965, after absence of exposure to trypsin for almost a year, the patient still manifested a significant decrease in vital capacity associated with the onset of asthmatic symptoms lasting one hour after nebulization of a 1.0 Gm. per cent trypsin solution. The patient claimed that this was the first wheezing he had experienced since he was discharged from his prior hospitalization in February, 1964. As noted in Table III, exposures to similar concentrations of trypsin solution in two asthmatic subjects with no history of trypsin contact led to no significant decrease in vital capacity nor precipitation of any asthmatic symptoms despite the induction of significant laryngeal and pharyngeal irritation in one subject.

Table III. *Provocative inhalation tests*

<i>Subject</i>	<i>Date</i>	<i>Substance nebulized</i>	<i>Vital capacity (ml.)</i>	<i>MMEFR* L./minute</i>
Patient J. R.	2-25-64	None	3,100	60
		Saline	3,280	60
		Trypsin, 1 mg.%	3,200	66
		Trypsin, 10 mg.%	3,500	60
		Trypsin, 100 mg.%	3,400	69
	2-26-64	Base line	2,700	44
		Trypsin, 1 Gm.%	1,500†	23†
		Isuprel, 2 minutes after	1,200	14
		Isuprel, 30 minutes after	1,000	18
		Isuprel, 60 minute after	2,100	32
	1-16-65	None	2,400	57
		Trypsin, 10 mg.%	2,600	34
		Trypsin, 1 Gm.%	1,800	21
Control subject No. 1	9-15-65	None	3,200	120
		Trypsin, 10 mg.%	3,600	90
		Trypsin, 100 mg.%	3,800	108
		Trypsin, 1 Gm.%	3,600	96
Control subject No. 2	3-18-66	None	3,200	204
		Saline	3,300	240
		Trypsin, 10 mg.%	2,400	246
		Trypsin, 100 mg.%	3,250	252
		Trypsin, 1 Gm.%	3,300	240

*MMEFR, maximum midexpiratory flow rate (middle 50 per cent of vital capacity).

†Acute dyspnea, wheezing, and coughing.

DISCUSSION

There has been little mention in the literature of adverse effects of aerosol trypsin therapy other than irritation of the upper respiratory tract.³ However, several cases of rhinitis and conjunctivitis in laboratory workers exposed repeatedly to trypsin and chymotrypsin powders have been reported.^{2, 5, 6} No asthmatic symptoms were reported in these few cases. It is of interest that the subjects of these reports and the patient studied by us all had strongly allergic backgrounds. We felt that the biologic powders to which our patient was exposed in his employment were significant offenders in the precipitation of his asthmatic symptoms. This was confirmed by provocation of asthma by inhalations of a saline solution of a trypsin preparation obtained from his place of employment. Although the patient claimed marked improvement after removal from any exposure to these biological preparations, the long-range prognosis must remain guarded in face of the persistent pulmonary function abnormalities.

Although there appears to have been a recent decrease in the use of trypsin aerosol therapy in bronchopulmonary conditions, it seemed of importance to report this adverse reaction to aerosolized trypsin because of several existent treatment programs involving the long-term use of nebulized trypsin solution in asthmatic patients.

The assistance of Dr. William F. Schoenwetter and the technicians of the Allergy and Immunology and Thoracic Clinics is gratefully appreciated.

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