

Papain-induced asthma—physiological and immunological features

Harold S. Novey, M.D., Louis E. Marchioli, M.D., William N. Sokol, M.D., and Ian D. Wells, B.Sc.(Tech.) Orange, Calif.

Increasing reports of respiratory disease associated with exposure to papain prompted clinical, physiological, and immunological studies of the supervisor of a meat tenderizer factory who developed asthma after long-term contact with papain dust. His symptoms were worse at work and better on weekends and vacations. Bronchial inhalation challenges produced both immediate and late asthma to papain but not to the other ingredients in the food product. Immunological studies revealed the presence of specific IgE antibodies by direct and passive transfer skin tests and the radioallergosorbent test, and specific precipitating antibodies by immunodiffusion tests. These findings are indicative of a dual type I and III hypersensitivity. Papain acting as an allergen in an occupational setting is a risk factor for eliciting asthma even in a nonatopic individual.

A growing list of products have been incriminated as provocative factors of asthma in an occupational setting.¹ Some of these, such as *Bacillus subtilis* extracts in enzyme detergents, have been shown to act as antigens. Others, like toluene diisocyanate, may act either as antigens or nonspecific irritants, while still others, including acetylene welding fumes, appear to be mainly irritants.²

Papain, a sulfhydryl protease of molecular weight 23,000 daltons, is derived chiefly from the unripe fruit of the papaya or pawpaw tree, *Carica papaya*.³ Its enzymatic properties have found application in the food and drug industries for such uses as clearing beer and cloudy contact lenses, tenderizing meat, lysing wound adhesions, treating Hymenoptera and jellyfish stings, and as additives in laxatives, tooth powders, digestive tablets, and skin lotions. Introduced into the lungs of laboratory animals (rats, hamsters, rabbits, dogs) by either endotracheal instillation or aerosol inhalation, it rapidly produces emphysematous changes and is used as a model of human emphysema.⁴ Reports of pathology in man appear limited to allergic reactions. Of the total 33 patients reported with one or

more disorders attributed to papain exposure (19 had asthma, 14 rhinitis, 8 urticaria and angioedema, 3 anaphylaxis),⁵⁻⁸ 4 were reported during the past year, and 2 involved a meat tenderizer product.^{9, 10} In the report from Canada, the same company's product was involved. The source in the English case has not been determined by us. In another, death from asthma and anaphylaxis occurred within thirty minutes of an accidental inhalation of papain powder by a sensitized worker.¹⁰

We present bronchial challenge and immunological studies for the first time in a case of papain-related asthma in order to define the pathogenesis.

CASE REPORT

The patient, a 58-yr-old white man who never smoked, developed increasing dyspnea, cough, and wheezing over a 2-yr period. His symptoms were most evident during work hours and subsided during vacations.

He had worked as supervisor of a meat tenderizer plant for twenty-two years. The products contained about 1% papain combined with sodium chloride and sugar in a powder. Certain varieties also contained garlic, paprika, tricalcium phosphate, caramel coloring, and vegetable oils. The plant had served mainly as a packager until ten years previously when crude papain latex received directly from the African sources was cleaned, milled, and mixed with the other ingredients at his plant. The average size of the papain particles present after grinding was 10 μ in diameter. The mixing process often discharged large clouds of powder in work areas and traces of powder were present throughout the three-story building.

Physical examination. The patient was tachypneic with

From the Department of Medicine, Division of Allergy-Immunology, University of California, Irvine.

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Reprint requests to: Harold S. Novey, M.D., Department of Medicine, California College of Medicine, University of California Irvine Medical Center, 101 City Drive South, Orange, CA 92668.

retractions of the supraclavicular and intercostal areas. There were generalized wheezes and rhonchi on auscultation of the lungs. A peak respiratory flow rate of 250 L/min (Wright peak flow meter) increased to 410 L/min following the subcutaneous administration of 0.3 mg of terbutaline. He was otherwise normal.

Laboratory examination. Complete blood count, SMA-12, urinalysis, VDRL, erythrocyte sedimentation rate, serum immunoglobulins including IgE, RA latex, and alpha-1 antitrypsin determinations, were normal. A chest film was interpreted as showing hyperinflation. Pulmonary spirometry done when the patient was less symptomatic after bronchodilator therapy showed normal FVC and FEV₁, slightly diminished FEV_{25%-75%}, and abnormal functional residual capacity (144% predicted) and residual volume (180% predicted). A single breath diffusing capacity was greater than predicted. Allergy skin tests to extracts of local pollens and common environmental agents were negative. Radioallergosorbent tests (RAST) for serum IgE antibodies to these allergens were also negative (Pharmacia Laboratories, Inc.)

Progress. Despite treatment with maximum doses of theophylline and beta adrenergic bronchodilators, the patient continued to experience daily episodes of coughing, wheezing, and dyspnea. Improvement but not complete remission resulted when corticosteroids were added. Despite his reluctance, he was forced to leave his job. His clinical condition gradually improved and his pulmonary function tests returned to normal on medication. Steroid therapy was discontinued after seven months and by one year his asthma was controlled by bronchodilators. He did not return to his former work and was awarded compensation for a work-related disability.

MATERIALS AND METHODS

Bronchial inhalation challenges. These were performed to help confirm a work-related disease, to identify the cause, and to determine whether the agent acted as irritant or antigen.

The initial challenge (No. 1) was designed to simulate work exposure, using techniques described by Pepys.¹¹ In a closed room the patient transferred three identical-appearing white powders containing lactose, food product without papain, and factory papain from one container to another. Particles were inhaled from the dust cloud that ensued. The following preparations for this and subsequent challenges were similar. Medications except for a short-acting aerosol bronchodilator were withheld for eight hours before. Flow volume curves (220 spirometer with XY recorder) were obtained in another area every 15 to 20 minutes until there was no more than a 10% change in the FEV₁ over a 1-hour baseline period. The baseline results were within at least 70% of his best previous effort. The pulmonary function tests (PFTs) were repeated 10 minutes after a challenge and at 10- to 30-minute intervals until a definite trend was established. These tests were performed over a 6-hour period in the laboratory. Two other subjects, one with allergic asthma, the other without asthma, served as controls.

Challenges Nos. 2-4. These were designed to study the

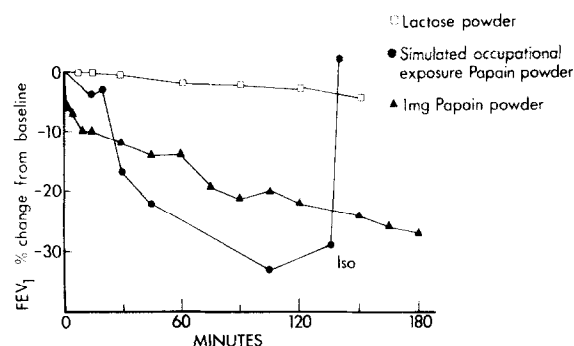


FIG. 1. Asthma reaction shown by decrease in FEV₁ on exposure to airborne papain powder (●) and the inhalation of 1 mg of papain powder (▲), but not to lactose powder (□) or food product without papain, not shown. Asthma was promptly reversed when the patient inhaled isoproterenol, 375 μg (ISO).

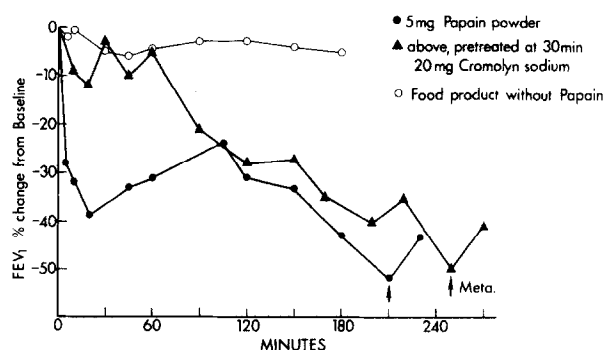


FIG. 2. An immediate asthma reaction shown by decrease in FEV₁ provoked by the inhalation of 5 mg papain powder (●) but absent when the exposure was preceded by the inhalation of cromolyn sodium, 20 mg (▲). The late drop in FEV₁ was unaffected. Inhalation of metaproterenol, 1.95 mg (↑), failed to completely reverse the reaction.

effects of measured amounts of the inhaled agents in their natural states. Capsules containing 200-mg amounts of either (1) lactose with factory papain or (2) the sugar and salt base without papain or (3) lactose powder alone were fitted into a hand-held, patient-activated turbo-inhaler (Spinhaler, Fisons) and used in a double-blind manner. The patient inhaled the contents of each capsule in about four deep inhalations. The capsules could not be distinguished by appearance or taste. In Challenge No. 2, 1 mg papain in 199 mg lactose was inhaled following the two control capsules. Challenges 3 and 4 used 5 mg papain with 195 mg lactose. The fourth challenge differed in that the patient was pretreated 30 minutes before challenge with 20 mg cromolyn sodium. Challenges 2, 3, and 4 were performed on separate days at least one week apart. Challenges with the nonpapain control substances were done randomly as expected in a double-blind test. A second challenge on the same day was done only if the first was negative after one hour. Exposures to the powders in air were 10 minutes for those that elicited no immediate response.

In order to determine if spontaneous asthma might occur

TABLE I. Physiological and immunological reactions to papain

<i>A. Bronchial challenge</i>				
	Immediate	Delayed asthma		
Low dose	+, reversed by bronchodilator	—		
Higher dose	+, blocked by cromolyn Na	+, not blocked by cromolyn Na or reversed by bronchodilator		
<i>B. Immunological</i>				
	Skin tests *		Serological tests	
	Direct	P-K	Serum IgE antibodies	Precipitins
Subject	+/+	+/+	+ †	+
Controls (n)	0/0 (2)	ND	0 (15)	0 (40)

*Results read at 20' / 5 to 7 hr.

†After absorption of precipitins.

TABLE II. RAST results for papain

	Patient	Control
Unabsorbed serum	290	320
IgG absorbed out	3,270	580
IgG, M, A absorbed out	6,990	570

Numbers are radioactive counts per 5 minutes above background; control counts vary due to dilutional effects on sera in absorption studies.

during the long laboratory visit without medication, the patient was examined when his baseline PFTs were within 10% of his challenge day values, and these tests were monitored every 30 minutes. Only after 8 hours was there a 20% decrease in PEFR or 15% drop in FEV₁.

IMMUNOLOGICAL STUDIES

Antigen preparations. Two forms of papain were used, designated crude (obtained from the patient's factory), and pure (Sigma, twice crystallized). These were dissolved in saline solution at doubling concentration from 1.25 mg/ml to 20.0 mg/ml. For skin tests, the solutions were passed through a Seitz filter. Heating at 100° C for 30 minutes did not alter their antigenicity in the tests applied. The sugar-salt base was prepared at 20 mg/ml and the commercial meat tenderizer at 10 mg and 20 mg/ml.

Direct and passive transfer (P-K) skin tests. These were performed on the subject's forearms with appropriate controls of diluent and histamine. A 1-10 dilution of the 10 mg/ml papain preparation was used for scratch, and 1-100 to 1-10,000 dilutions for intradermal testing. The recipient for the P-K test was the patient's wife, who did not react to papain by direct skin testing. The patient's serum was nega-

tive for hepatitis antigen by ragweed radioimmunoassay (RIA). Both unaltered and serum heated at 56° C for one hour were used. Skin test readings were done at 20 minutes, 5 to 7 hours, and 24 hours. Two persons not known to be exposed to papain served as controls.

Radioallergosorbent tests (RAST). In order to detect specific IgE antibodies to papain in sera, conjugates were prepared by coupling 25 mg amounts of antigen to 100 mg of cyanogen bromide-activated cellulose (microcrystalline, Merck). Suspensions of 0.5 ml containing 1.0 mg of the conjugate were added to 0.1 ml aliquots of serum and monitored in an auto-gamma counter for ability to bind anti-IgE ¹²⁵I (Pharmacia Laboratories, Inc.).¹²

Test serum was either neat or treated to remove precipitating antibodies by repeated additions of rabbit antihuman IgG or IgG, M, A (Hyland) and removal of precipitants by centrifugation. There were still traces of precipitin arcs present in the absorbed serum. Such treatment was used to remove possible competing antibodies that would interfere with the RAST.¹³

Controls consisted of 15 sera of normal persons not known to be exposed to papain. Both treated and untreated sera were used.

Precipitin tests. Double-diffusion tests were performed using an agar gel Ouchterlony method.¹⁴ Control sera of persons (40) with no prior history of factory exposure to papain were used.

RESULTS

Bronchial inhalation challenges

The results are depicted in Figs. 1 and 2 and summarized in Table I. Under simulated work conditions, the patient developed asthma after three minutes' exposure to papain-laden air. The asthma was reversed after he inhaled 375 µg of isoproterenol. Neither of two control subjects simultaneously exposed had any response.

The exposure to 1 mg papain by Spinhaler also produced only immediate asthma but to a milder degree. The response to 5 mg papain (Fig. 2) was more intense. There was a 28% drop in FEV₁ at 5 minutes followed by gradual spontaneous improvement reaching a maximum increase of 15% at 105 minutes. This was followed by a second phase of gradual deterioration until the FEV₁ fell to 48% of baseline. This time treatment with a comparable dose of bronchodilator as in the prior challenge produced only a modest response. The immediate but not the delayed reaction was ablated by premedication with cromolyn sodium under similar baseline conditions one week later (Fig. 2). There was no response to inhalation of lactose or food base without papain in any of the challenges.

IMMUNOLOGICAL STUDIES

Direct and passive transfer (P-K) skin tests

The patient had strongly positive immediate reactions to both prick and serial intracutaneous (IC) dilu-

TABLE III. Double diffusion test results to papain and patient's sera

Dates	Number of precipitin arcs above controls at papain concentration (mg/ml) of:				
	20	10	5	2.5	1.25
12/17/75	8	8	8	7	7
1/15/76	8	8	8	7	7
3/18/76*	8	8	8	7	7
4/15/76	ND	ND	ND	6	6
11/4/76†	4	4	4	5	4

*Terminated employment and papain exposure.

†Return to normal pulmonary function and off steroid medication.

tions of the antigens on three occasions six months apart (Table I). At seven hours there were erythematous edematous reactions at the IC sites with a direct relation between reaction size and dose.

The serum passive transfer tests produced similar appearing immediate and 5 hour later reactions. There was a near ablation of the immediate reaction with heated sera. The late reaction was unchanged. Two control subjects had negative direct tests to the papain antigens.

RAST

There was no difference in values between the patient and controls when this test for IgE antibodies to papain was applied to unabsorbed sera. There were highly significant differences, however, when either IgG or IgG, M, and A was partially removed (Table II). The 15 control sera were negative for IgE antibodies to papain. Neither patient nor control sera reacted to the meat tenderizer without papain.

Precipitin tests

The patient's sera contained multiple precipitating antibodies at all five antigen concentrations while controls were negative (Fig. 3). The precipitins decreased after the patient's removal from the factory (Table III).

DISCUSSION

The subject developed asthma associated with exposures to the proteolytic enzyme papain. This relationship was suspected by the physician trained in allergy during the patient's first visit, because he elicited the history of improvement in symptoms when the patient was away from work.

Immunological reactivity appears to be a contributory factor in the pathogenesis of our patient's asthma. He had specific IgE antibodies to papain in abundance as judged by both the direct and passive

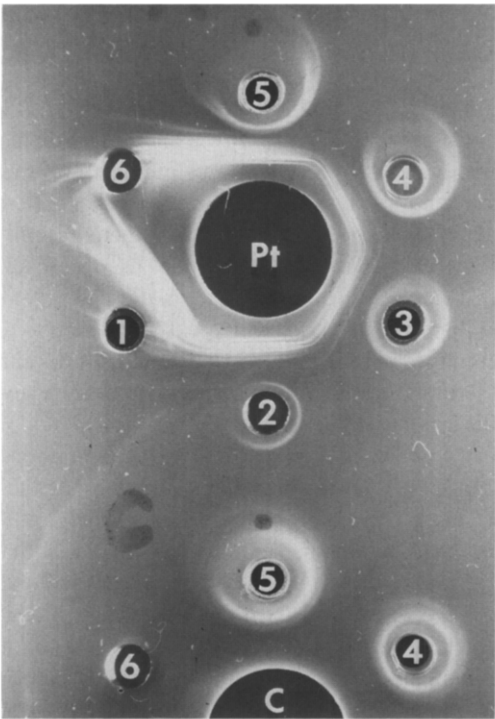


FIG. 3. Double-diffusion test in agar gel. Patient and control sera, undiluted, are in the central wells; papain extract in concentrations of 1.25, 2.5, 5.0, 10.0, and 20.0 mg/ml is in wells 1 to 5, respectively, and food product without papain (20 mg/ml) is at 6. Multiple lines are present to papain and patient's serum and not to control serum or to food product base. Concentric lines around wells with higher papain concentrations are nonantibody and probably represent an enzyme-antienzyme reaction.

transfer skin tests and RAST results. Asthma was provoked immediately and specifically by the inhalation of papain. The severity was dose-related. The immediate reaction was completely reversed by a bronchodilator and abolished by pretreatment with cromolyn sodium. These findings are consistent with a classical IgE antibody-mediated reaction. The patient, however, also produced precipitating antibodies and had a dual asthma response to challenge at higher doses of papain.

Such dual reactions are similar to those found with *Aspergillus* and various occupational allergens.¹¹ Precipitating antibodies acting in immune complexes may be the pathogenic agents, although they have a more established relation with pulmonary interstitial disease than asthma. Dolovich and his associates¹⁵ believe that IgE antibodies can participate in eliciting late clinical reactions, and Solley and co-workers¹⁶ have evidence of late skin reactions by IgE antibody. The fact that the patient required steroid therapy to control his asthma and that precipitin activity has decreased in concert with clinical improvement is sug-

gestive but indirect evidence of a type III or immune complex reaction for the delayed asthma.

Although asthma is associated with papain exposure in man, the pulmonary emphysema so readily produced in the laboratory was not present in our patient, at least by clinical and pulmonary function criteria, and has not been reported in others. Particle size may have prevented sufficient penetration into the lower airways. Animal studies used aerosolized solutions or particles of 1 to 3 μ in diameter. Species variability may be another factor. Cats tended to resist the emphysematogenic effect of papain.¹⁸ An antipapain serum factor varied quantitatively among several animal species.¹⁸

An intriguing immunological explanation for this resistance to emphysema was proposed by Milne and Brand,⁷ who speculated that an inhibitory antibody might exist in human sera similar to that found in animal experiments. Arnon and Shapira,¹⁹ with whom one of the authors (H.S.N.) collaborated,²⁰ studied the interaction of papain, its substrates, and rabbit antipapain antibodies to establish the relation between binding sites of antibody and substrate on a protein molecule. They found that some precipitating antibodies inhibited the enzymatic activity by steric hindrance with the active site. Thus, the precipitins found in this patient, while possibly contributing to the pathogenesis of late asthma by an immune complex mechanism, might also protect against the emphysemic potential of papain.

Nonspecific serum protein factors must also be considered. Human α_2 macroglobulins have an antienzyme effect,²¹ while human α_1 antitrypsin, when applied intratracheally, has been shown to inhibit papain-induced emphysema in hamsters.²²

It appears surprising that the patient did not exhibit asthma until some 20 years after working with meat tenderizer. For the first ten years, however, he was exposed mainly to the finished product in a packaging and distribution capacity. Afterwards, raw papain was milled and mixed at his plant. The average particle size of the papain dust at 10 μ means that nearly all could be lodged in the nasal and oral cavities, and relatively little would reach the bronchi.¹⁷ Such lack of accessibility to the target area is one explanation for the late appearance of asthma and another may be the patient's relatively unresponsive IgE system.

Papain exposure is a health hazard to workers as an antigen-provoking allergic asthma even in nonatopic persons. Precautions should include effective air cleaning devices and respiratory masks at work sites, milling of powder to particle sizes well over 20 μ in diameter, screening of atopic and asthma employee

applicants and those with α_1 antitrypsin deficiencies, and monitoring of exposed workers with examinations that include an assessment of bronchial obstruction and skin or immunoserological tests to papain. In this case, the patient's employers have instituted some air cleaning measures but were unwilling to study the effect of their product on the health of the other employees. Management representatives expressed concern about the effect of unfavorable publicity on the sale of their product. We are further persuaded that this enzyme is a serious industrial hazard by ongoing investigation of another factory where nearly all exposed employees have respiratory symptoms and immunological responses to papain. Risks to the consumer by papain-containing products are virtually unexplored.

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