

Papain-induced allergic reactions

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Summary

Papain is a proteolytic enzyme with elastolytic activity, which produces emphysema-like lesions when introduced into the airways of animals. It is encountered by humans in numerous occupations, medications and domestic products.

Sensitization to papain in two subjects in different occupations was confirmed with skin tests with chemical-grade papain and radioallergosorbent tests (RAST), with a papain which had been chemically inactivated by selective active site blockade. Skin tests and RAST were negative in non-symptomatic co-workers. Withdrawal from occupational exposure to papain resulted in an improvement in symptoms.

In a survey of 330 subjects at the time they were receiving routine allergy skin tests, seven reacted to papain. Sensitization was confirmed by the RAST. Serum IgG antibodies to papain were detected among sensitized individuals and also in five out of 266 sera obtained from a clinical hospital laboratory.

The findings illustrate immune responses to papain in humans in the form of atopic sensitization and serum IgG antibodies. Moreover, recurrent respiratory symptoms in the presenting persons served to illustrate that they and their co-workers were repeatedly exposed to air-borne papain. In view of the potential danger from the proteolytic effects of papain, these observations illustrate the need for further investigations of the effects of human exposure to air-borne papain.

Introduction

Papain is a proteolytic enzyme, molecular weight 23,000, derived from *Carica papaya*. It is made up of 198 amino acids in a single polypeptide chain with three disulphide bridges and an active sulphhydryl group. It has a wide enzymatic specificity against protein and low molecular weight substrates (Smith, 1967).

Papain produces emphysema-like lesions in experimental animals of numerous species when introduced into the airways by intratracheal instillation or by aerosol

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inhalation (Gross *et al.*, 1964; Giles, Finkel & Leeds, 1970; Johanson, Pearce & Reynolds, 1971; Kilburn, Dowell & Pratt, 1971; Pushpakom *et al.*, 1970). There is as yet no documentation of the production of emphysema in man.

Allergic reactions to papain have been reported since 1928, when Eyermann described a druggist with rhinitis produced by Caroid, a proprietary name for papain (Eyermann, 1928). Asthma induced by papain has been reported in only six cases (Beecher, 1931; Osgood, 1945; Milne & Brand, 1975).

This study was performed to further document, with *in vitro* and *in vivo* tests, the occurrence of papain-induced sensitization and symptoms.

In addition, the tests were used as indices of the prevalence of immune responses to papain in populations with no known occupational exposure. A review of the numerous sources of potential exposure to air-borne papain is presented.

Case reports

Case 1

A 24 year old Red Cross blood bank laboratory technician presented in January, 1975, with persistent itchy and watery eyes for 14 months. Prior to this, she had had flexural eczema in childhood, up to the age of 18 years, and perennial sneezing, rhinorrhoea and variable dyspnoea, with wheezing and chest tightness, since the age of 20 years. The symptoms were provoked by cats, exercise, cold air, smoke and dust. She was only exposed to cats infrequently and had never smoked.

Physical examination was normal, apart from some swelling of the nasal mucosa.

Spirometry was normal, the forced expired volume in one second (FEV₁) and vital capacity (VC) being 3.2 and 3.85 litres respectively (predicted 2.95 and 3.6 litres). A chest radiograph was normal. A histamine inhalation test was carried out by a method which has been described in detail (Killian *et al.*, 1976; Cockcroft *et al.*, 1977) to determine the degree of non-specific bronchial reactivity. Inhalation of histamine acid phosphate, 8 mg/ml for 2 min, produced a fall in FEV₁ of 15%. This level of reactivity is within the normal range determined by a large population study in this laboratory (Cockcroft *et al.*, 1977). The blood eosinophil count was raised at 547/mm³, and the α_1 -antitrypsin level was within the laboratory normal range at 216 mg/dl (normal range 180–380 mg/dl). Prick tests with a group of seventeen common allergen extracts gave weal and flare responses to cat and house dust extracts.

No relation of her symptoms to specific exposure at work was initially recognized. She subsequently noted that her upper respiratory symptoms were worse when she handled small quantities of papain powder in order to prepare a solution for incubation with red blood cells. She prepared the solution only at 6-month intervals, but used the solution every day. She had been exposed to papain for a total of 2 years.

She then moved to the main floor of the laboratory, where papain was not used, and her symptoms improved within 1 month. When she returned to work on the second floor, where she was exposed to papain, the symptoms returned within 3 days. She is now restricted to the main floor. Mild perennial nose and eye symptoms have persisted, but she is much improved and has no exacerbations of these symptoms.

Case 2

A 27 year old man was seen in December, 1975. Since January, 1973, he had experienced three episodes of watering, runny and itchy eyes, a stuffy nose, non-productive

cough, wheezing and itching over the skin of the back of the neck. On each occasion, the symptoms were initially severe enough to require treatment with adrenalin, and they kept him off work for a few days and led to episodes of wheezing for up to 3 weeks. The acute episodes began while he was at work.

He had worked since 1965 as a mechanic in a commercial packaging plant handling a variety of powders. The first two attacks in 1973 occurred while the firm was packaging a douche powder containing papain. The third attack occurred in 1975 during the packaging of a papain-containing meat tenderizer. The patient had been intermittently exposed to the douche powder since 1965 and the meat tenderizer since 1974. The products were packaged two or three times a year for about 2 weeks at a time. In the first attack, the symptoms developed after 2 or 3 consecutive days of exposure to the douche powder, but the subsequent attacks occurred on the first day of exposure.

The patient was otherwise well. There was no past history of other atopic or chest disease. He had smoked ten to twenty cigarettes a day for 13 years, but denied chronic cough, sputum or effort dyspnoea.

On physical examination, there was mild nasal mucosal swelling, expiratory rhonchi and prolonged forced expiratory time.

Investigations were carried out three weeks after the last acute episode. A chest radiograph was normal. The FEV₁ and VC were normal, but there was a reduced maximum mid-expiratory flow rate (MMEFR) and increased residual volume (RV), indicative of small airways obstruction, and there was a reduction in D_LCO suggesting ventilation and perfusion imbalance (Table 1). The blood eosinophil count was 244/mm³. α_1 -Antitrypsin was below the laboratory normal range at 160 mg/dl (normal range 180–380). Prick tests with seventeen common allergen extracts were negative.

After December, 1975, he continued to work at the packaging firm. He has avoided exposure to papain and remained symptom-free. Repeat pulmonary function tests in November, 1976, showed a return of the MMEFR to normal and an improvement in

Table 1. Case 1: pulmonary function tests

Test	Predicted (mean $\pm$ s.d.)	Observed		
		January 1976	November 1976	February 1977
FVC (litres)	4.55 $\pm$ 0.74	4.85	4.9	
FEV ₁ (litres)	4.00 $\pm$ 0.55	3.70	3.6	
FEV ₁ /VC (%)	88	76	73	
MMEFR (l/sec)	4.3 $\pm$ 1.12	3.36	4.20	
Slow VC (litres)	4.55 $\pm$ 0.88	4.65	4.96	
FRC (litres)	3.28 $\pm$ 0.62	4.54	5.03	
RV (litres)	1.50 $\pm$ 0.39	2.63	2.60	
TLC (litres)	6.14 $\pm$ 0.68	7.28	7.50	
Respiratory frequency		15	12	
Tidal volume (litres)		0.64	0.47	
D _L CO (ml/min/mmHg)				
(steady state)	22.9 $\pm$ 2.1	11.2	12.4	
Fractional uptake CO (%)	52	36.3	47	
Static compliance (l/cmH ₂ O)	0.2–0.4			0.25
P _{e1} max. (cmH ₂ O)	36			38

fractional uptake of carbon monoxide (Table 1). Static compliance of the lungs (P_{el} max.) was normal, thus providing no evidence of emphysema. A histamine inhalation test, performed by the same method as for case 1, showed a moderate increase in non-specific bronchial reactivity; inhalation of histamine acid phosphate, 1 mg/ml, for 2 min produced a fall in FEV_1 of 27%.

Materials and methods

Papain, for skin and *in vitro* tests, was obtained from the Sigma Chemical Company (St Louis, Missouri, U.S.A.; P3125, lot No. 16C-8090), in the twice-crystallized form suspended in 0.05 M sodium acetate, pH 4.5, containing 0.1% thymol. It contained 18 u/mg of proteinase (1 u is defined as the amount of enzyme which hydrolyses 1 μ mol of alpha-N-benzoyl-L-arginine ethyl ester (BAEE) per min at pH 6.2 at 25°C).

Prick tests (Pepys, 1975) were performed with a solution of 10 mg/ml papain in phosphate-buffered saline. Subjects with a history which suggested sensitization were tested initially with 0.1 and 1 mg/ml. The diluent control was used in separate sites and the skin tests were read after 15 minutes. A positive test consisted of a weal at the papain test site in the absence of a similar response at the diluent site.

In vitro immunological tests were performed with papain, which was rendered inactive by blocking the active enzyme site with the irreversible chemical inhibitor, chloromethyl ketone of lysine (TLCK). The latter does not interfere with antigenicity, but acts specifically on the active centre of papain, eliminating the enzymatic activity (Whitaker & Perez-Villasenor, 1968), which may otherwise interfere with *in vitro* tests (Dolovich & Little, 1972; Wicher & Dolovich, 1972; Pepys *et al.*, 1973). A solution of inactivated papain containing 11.16 mg protein per ml, as estimated by the Lowry (1951) technique, was prepared. The preservation of antigenic reactivity was initially confirmed in precipitin tests with rabbit antisera to papain. Radioimmunoassay to detect papain-specific IgG antibodies was performed by methods previously described (Dolovich *et al.*, 1970). Briefly, antiserum to human IgG was placed in the centre wells and 2 μ l aliquots of serum in the surrounding wells, followed by addition to the centre well of 10 μ l of a 1:10 dilution of ^{125} I-labelled papain. The radioallergosorbent test (RAST) for the detection of IgE antibody was performed as previously described (Ceska, Eriksson & Varga, 1972). Activated cellulose discs were exposed to inactivated papain, 11.16 mg/ml, for coupling.

A positive RAST was a result greater than 2 s.d. above the mean for the sera of skin test-negative subjects.

In addition to cases 1 and 2 with clinical sensitivity to papain, three groups of subjects were studied. One consisted of eighteen asymptomatic Red Cross personnel working in the same building and with the same papain exposure as case 1. A second group was 330 consecutive patients receiving allergy skin tests in a Chest and Allergy Clinic. The third group was 231 patients whose blood happened by chance to be sent to a clinical hospital laboratory on one or other of 2 days that specimens were obtained.

Results

Cases 1 and 2 had positive prick tests with papain. Case 1 developed a 9 mm weal in response to 10 mg/ml. Case 2 developed weals with 8 and 12.5 mm diameters in response to 0.1 and 1.0 mg/ml respectively. Serum IgE (Fig. 1) and IgG (Table 2) antibodies to papain were demonstrated in both patients.

Table 2. Serum IgG antibodies to papain

RIEP* for IgG antibodies	Blood bank employees, papain skin test:		Clinic, papain skin test: positive	Random samples from clinical laboratory (skin test not done)
	Negative	Positive		
Negative	17	0	0	226
1+	1	1	2	2
2+	0	0	2	3

* Radioimmuno-electrophoresis.

Prick tests with papain, 10 mg/ml, were performed in eighteen Red Cross laboratory co-workers of case 1 and in 230 clinic patients receiving routine allergy skin tests. In the workers, no additional positive skin tests were found. In the group of allergy clinic patients, excluding case 2, seven out of 330 (2.1%) had positive skin tests. None of these seven had a history of papain-induced symptoms; two could recall using papain in powder form as a meat tenderizer in the home.

RAST results are summarized in Fig. 1. The test was positive in the symptomatic sensitized blood bank employee, but negative in fourteen unsensitized co-workers from whom serum samples could be obtained by the time of the test. It was also

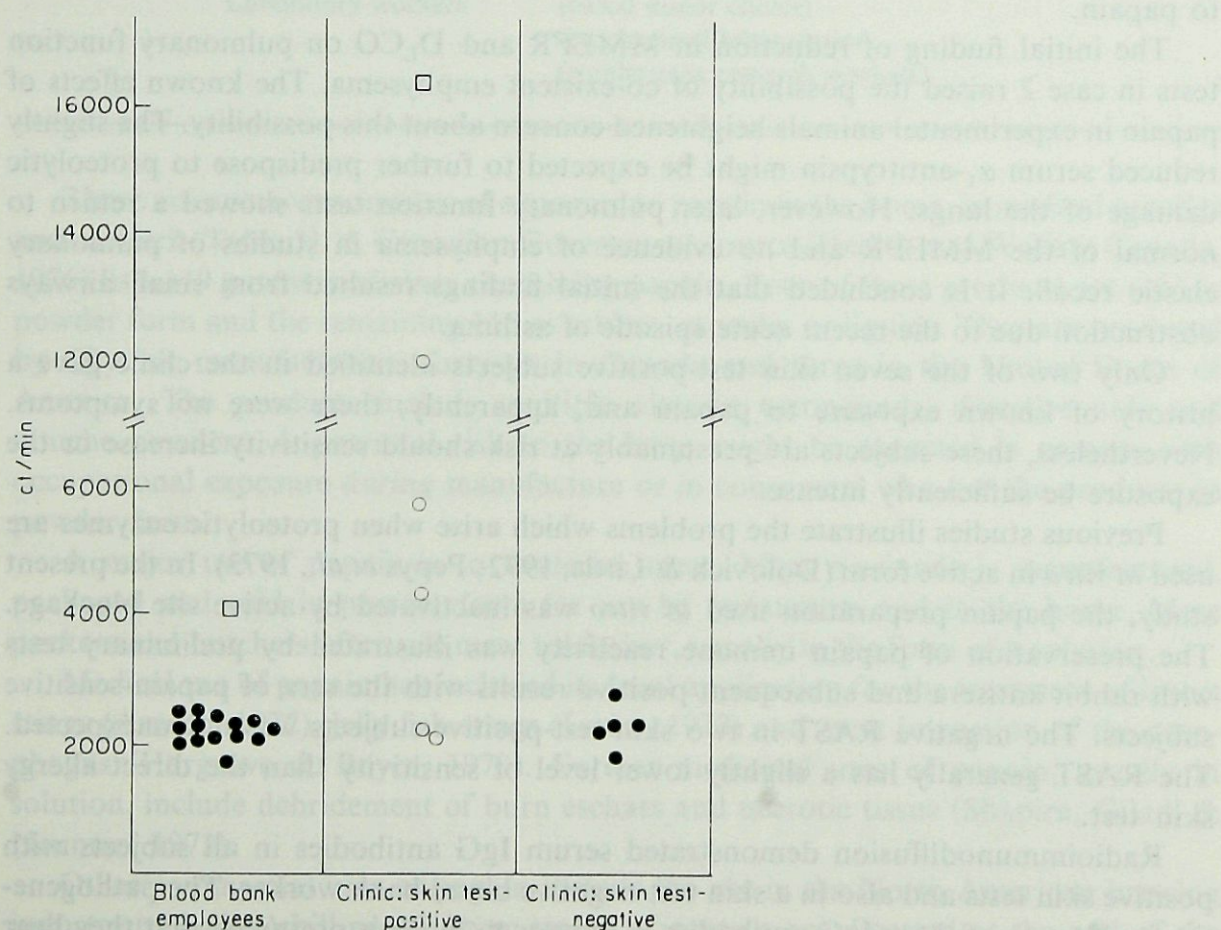


Fig. 1. Radioallergosorbent test (RAST) with chemically inactivated papain: (●) skin test-negative subjects; (○) skin test-positive patients, in whom there is no knowledge of symptoms relating to papain; (□) symptomatic skin test-positive patients, the symptomatic skin test-positive patient in column 1 is case 1 and in column 2 is case 2. The RAST confirms the presence of IgE-dependent sensitization in the skin test-positive persons.

positive in four of six skin test-positive clinic patients on whom serum samples were obtained, including case 2. Skin test-negative clinic patients served as controls.

The results of RIEP for serum IgG antibodies to papain are shown in Table 2. The test was negative in seventeen out of nineteen Red Cross blood bank workers and was positive in one sensitized worker (case 1) and one non-sensitized worker. It was also positive in all four skin test-positive clinic patients, including case 2. Serum was not available from the four skin test-positive clinic patients. The test was positive in 2.1% of blood specimens obtained at random from a clinical laboratory.

Discussion

Case 1 developed only upper respiratory symptoms on contact with papain, despite a history of asthma provoked by other factors. Non-allergic bronchial reactivity, as measured by a histamine inhalation test, was normal (Cockcroft *et al.*, 1977). Thus the induction of asthma would require an especially intense allergic reaction in the airways (Killian *et al.* 1976). This, in turn, appears to be a function of the level of exposure to air-borne antigen and the level of sensitization. In case 2, the moderately increased non-allergic bronchial reactivity supports the diagnosis of asthma. In both patients the observed relationship of symptoms to papain exposure, the improvement associated with withdrawal from exposure and the demonstration of sensitization and IgE antibodies to papain are strong support for the diagnosis of symptomatic allergy to papain.

The initial finding of reduction in MMEFR and D_LCO on pulmonary function tests in case 2 raised the possibility of co-existent emphysema. The known effects of papain in experimental animals heightened concern about this possibility. The slightly reduced serum α_1 -antitrypsin might be expected to further predispose to proteolytic damage of the lungs. However, later pulmonary function tests showed a return to normal of the MMEFR and no evidence of emphysema in studies of pulmonary elastic recoil. It is concluded that the initial findings resulted from small airways obstruction due to the recent acute episode of asthma.

Only two of the seven skin test-positive subjects identified in the clinic gave a history of known exposure to papain and, apparently, there were no symptoms. Nevertheless, these subjects are presumably at risk should sensitivity increase or the exposure be sufficiently intense.

Previous studies illustrate the problems which arise when proteolytic enzymes are used *in vitro* in active form (Dolovich & Little, 1972; Pepys *et al.*, 1973). In the present study, the papain preparation used *in vitro* was inactivated by active site blockage. The preservation of papain immune reactivity was illustrated by preliminary tests with rabbit antisera and subsequent positive results with the sera of papain-sensitive subjects. The negative RAST in two skin test-positive subjects was not unexpected. The RAST generally has a slightly lower level of sensitivity than the direct allergy skin test.

Radioimmunodiffusion demonstrated serum IgG antibodies in all subjects with positive skin tests and also in a skin test-negative blood bank worker. The pathogenic significance of these IgG antibodies is unknown, but it is presumed that they bear no relationship to pulmonary or systemic disease and are merely an indication of exposure to papain. The test for IgG antibodies to papain was positive in 2.1% of the clinical laboratory blood samples, suggesting that exposure to papain among members of the general public is not unusual.

Table 3. Sources of exposure to papain

Class	Source
Domestic	Patent digestive aids and vitamin compounds Douche powder Meat tenderizer <i>Carica papaya</i> fruit and juice
Medical	Insect stings Jelly fish stings Meat impaction of the esophagus Ulcers and necrotic tissue debridement
Occupational	Digestive aids (manufacturers and packers) Douche powder (manufacturers and packers) Meat tenderizer (manufacturers, packers and distributors of the powder) (meat packers) (butchers) (home freezer meat dealers) (restaurants) Beer chill-proofing factor (manufacturers) (brewers) Bating enzyme (manufacturers) (tanners) Laboratory workers (blood donor clinics) (biochemical laboratories) (emphysema research workers)

There are numerous sources of exposure to papain in the home, in medical practice and at work (Table 3). A Canadian Government source (Health and Welfare Canada, 1976) lists 119 patent medicines containing papain. Four of these products are sold in powder form and the remaining 115 as tablets, capsules or liquids. They are produced by fifty-five manufacturers, forty-six in Canada and three in the United States of America. The products include multiple vitamin compounds, digestive aids and douche powders. A potential risk to the lungs might be expected in persons with occupational exposure during manufacture or in consumers who use the products in powder form.

A major use of papain is to tenderize meat. Meat marinade is manufactured, packaged and sold in powder form for use by restaurants and in the home. Meat packers and butchers often use meat tenderizer, usually in the form of a solution.

Medical use of papain has included its local application for the treatment of insect stings (Arnold, 1972), jelly fish stings (Loder, 1973) and meat impaction of the oesophagus (Hargrove & Boyce, 1970). Further medicinal uses of papain, usually in solution, include debridement of burn eschars and necrotic tissue (Shapira, Giladi & Neuman, 1973).

Other commercial uses of papain include the use in the North American brewing industry to make 'chill-proof' beer: traces of protein are digested at the end of the brewing process to prevent turbidity on cooling (Balls, 1941). It has been used in the tanning of skins and hides (Merck's Index, 1940). The latex of *Carica papaya* has previously been used in chewing gum manufacture (Hill, 1937). Caroid tooth powder, containing papain, has been a source of close exposure to papain powder and

sensitization (Osgood, 1945). Papain is also used as a proteolytic enzyme in biochemistry and haematology laboratories and to produce experimental emphysema in animals (Gross et al., 1964; Giles et al., 1970; Johanson et al., 1971; Kilburn et al., 1971; Pushpakom et al., 1970).

The capacity of papain, introduced into the airways, for inducing emphysema has been demonstrated in a variety of species, including rats, hamsters, rabbits and dogs. The dose of papain introduced into the airways experimentally has generally exceeded 1 mg. It is not yet known whether equivalent levels of exposure to air-borne papain occurs in humans and there is, as yet, no demonstration of human emphysema due to papain.

It would, nevertheless, be prudent at the present time to consider that individuals allergic to air-borne papain in occupational settings might be serving a function analogous to the canary in the coal mine. The respiratory symptoms of the allergic individual call attention to the presence of air-borne papain which may, in fact, be being inhaled by all persons in the particular environment. There is a potential risk of irreversible lung disease from the enzymatic effects of inhaled papain in exposed workers. These considerations illustrate the need for additional studies of the problem.

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