

Asthma due to inhaled chemical agents: ampicillin, benzyl penicillin, 6 amino penicillanic acid and related substances

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Summary

Three out of four workers from an antibiotic-producing factory developed late asthma and eosinophilia on inhalation challenge testing with ampicillin and related substances. The chemicals to which each worker reacted varied, suggesting differences in the clinically important allergens for each individual.

The taking by mouth of ampicillin in one patient, and benzyl penicillin in another in therapeutic doses led to the development of late asthma accompanied by skin and gastro-intestinal disturbances.

The inhalation of 200 µg of beclomethasone dipropionate before in one case, and before and 3-hourly after provocation challenge in the other, blocked the late asthmatic reaction. 40 mg sodium cromoglycate similarly given failed to inhibit the reactions. In one patient, 0.16–0.24 mg of isoprenaline sulphate inhaled 10 min before or 60 min after inhalation testing prevented the development of late asthma, and when given during the asthmatic reaction led to its rapid reversal.

Introduction

Shortly after the introduction of penicillin for the treatment of bacterial infections in man, allergic reactions were found to occur with a frequency of 1.2 to 5.2% (National Research Council, 1943; Lepper *et al.*, 1949). These reactions were more common after parenteral than oral administration and were most often manifest as urticaria or angio-oedema (Chancery, 1955). Similarly, skin rashes have been observed following treatment with ampicillin, the widest used semisynthetic penicillin, with a prevalence as high as 9% (Boston Collaborative Drug Surveillance Program, 1973).

Asthma occurring alone as a manifestation of allergy in patients receiving these drugs has not been reported, though it may occur as part of generalized anaphylaxis (Willis & Phair, 1970). Precisely what the relevant allergen is in these reactions remains unclear, though it does appear that there is no single substance which will account for all the allergic phenomena, but rather a large number of antigenic compounds, some clinically more relevant than others (de Weck, 1968).

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This paper reports the occurrence of asthma in three out of four workers employed in the production of the semisynthetic penicillin antibiotics. Factory exposure to ampicillin and compounds related to its synthesis was by skin contact and inhalation of the fine dusts of these materials during the manufacturing process. These asthmatic patients were investigated by inhalation challenge testing using the method previously described for industrial dusts (Pepys, Pickering & Loudon, 1972c). The clinically important allergens for each worker were shown by inhalation testing with ampicillin and its related compounds, some in more highly purified forms, as well as with possible impurities.

Although exposure to ampicillin and related compounds occurred in these workers by contact and inhalation of fine dusts, it was clearly important with regard to future medication, to discover the effect of oral ingestion of penicillin and ampicillin, and the results of this type of provocation challenge are described.

Materials and methods

Patients

Four males, age range 27–45 years, employed in a factory producing penicillin and a number of semisynthetic penicillin antibiotics, including ampicillin were studied. They had been working in the factory for 3–12 years. Three developed asthma and rhinitis 2 years after starting work with these materials, and of these, two also suffered from episodes of eczema. The fourth developed productive cough and shortness of breath 4 years after first contact with ampicillin and related compounds. All were admitted to hospital for investigation.

Skin tests

Prick tests were made with a battery of 23 common inhalant and food allergens, as well as with solutions of ampicillin 10^{-2} M, ampicillin polymer 10^{-6} M, benzyl penicilloyl polylysine 10^{-6} M, minor determinant mixture 10^{-2} M, and *E. coli* amidase solutions at concentrations of 1 to 10% w/v.

Body temperature, eosinophil and neutrophil polymorphonuclear leucocyte counts

Oral body temperature was taken before, 1 hr and 8 hr after each provocation test. Similarly, eosinophil and neutrophil polymorphonuclear leucocyte counts were performed from blood taken before, 8 and 24 hr after the tests.

Inhalation challenge tests

Materials. The following were used. 'Analar' lactose as the control and as the vehicle for the test materials. Also used were commercial ampicillin and commercial 6 amino penicillanic acid, the preparation of which involved the use of *E. coli* amidase, and commercial benzyl penicillin. Further test materials were extracts of *E. coli* amidase, benzyl penicilloyl polylysine and 6 amino penicillanic acid impurities. Samples of commercial 6 amino penicillanic acid, referred to as 6APA and commercial ampicillin which had been further purified by an absorption process to remove high molecular weight protein impurities (Munden, 1965), were used and referred to as 'purified' 6APA and 'purified' ampicillin. Ampicillin manufactured from penicillin by a partly chemical method not involving the use of *E. coli* amidase enzyme (Gist-Brocades, 1967) was used, and will be referred to as ampicillin (chemical) to distinguish it from ampicillin (commercial) prepared by the use of *E. coli* amidase.

Method. The investigation of all patients began with a control challenge using the lactose vehicle alone. The lactose was dried by heating overnight at 105°C, placed in two plastic trays, and then simply tipped backwards and forwards from one tray to the other 0.3 m below. This manoeuvre was performed in a confined space for 30–60 min (Pepys *et al.*, 1972c). During the test the patient wore a disposable surgical cap, gown and gloves, which were discarded at the end of the challenge test whilst the dust was being extracted. This reduced contamination of the patient's clothes and skin by the materials, making it less likely that continuing exposure would accidentally occur after completion of the provocation challenge. Provocation challenge with the test material was carried out in exactly the same way with a given amount of the substance added to the lactose vehicle. Since we had no prior experience of challenge testing with ampicillin and related substances, it was necessary to start with very small amounts, namely 10 mg added to 250 g lactose. The amount was increased by tenfold steps until a measurable reaction occurred. Definite asthmatic reactions were obtained by using 1–10 g of ampicillin, 6APA or benzyl penicillin added to the lactose. Since all the test materials were in the form of fine white powders, the patients were unable to distinguish on this account control from test challenges.

Respiratory function was monitored using a dry spirometer (Vitalograph®) to measure FEV₁ and FVC, and a Wright's meter to measure peak expiratory flow rate. The measurements were made before challenge, every 10 min after the challenge for the first hour, and then hourly for the rest of the day, and when necessary during the evening and night. There were no immediate asthmatic reactions but the provocation challenge testing could have been terminated if clinical features or measurements indicated that a definite bronchial reaction had occurred.

Oral challenge tests

Methods. The patient swallowed two capsules containing in total 500 mg of commercial ampicillin or benzyl penicillin. In each case this was a sample of the same material used in the inhalation challenge tests.

Pharmacological agents (Table 4)

The effects on the induced asthmatic reactions of inhaled isoprenaline sulphate 0.16–0.24 mg, sodium cromoglycate 40 mg, and beclomethasone dipropionate 200 µg were studied in patients 1 and 2. Sodium cromoglycate and beclomethasone dipropionate were given 30 min before challenge testing and also, in patient 1, 3-hourly thereafter for 12 hr. In patient 2, isoprenaline was administered on separate occasions 10 min before challenge, 1 hr after allergen challenge and during the actual asthmatic reaction. The result of administration of sodium cromoglycate and beclomethasone dipropionate both before and 3-hourly after challenge with these materials has been reported (Pepys *et al.*, 1974).

Case histories

Patient 1, male, aged 39 years a maintenance engineer, began working with ampicillin and related compounds at the age of 33 years. His particular job involved working in many different areas of the factory. After 2 years he developed sneezing and wheezing. Although he came into contact with many different compounds, he thought that his symptoms were particularly related to contact with ampicillin. Fifteen to 30 min after such an exposure he would develop wheezing and sneezing, lasting for about

1 hr, following which he would improve, only to awake in the early hours of the following morning with cough and tightness in the chest. It was these late symptoms that were his chief complaint. He noticed that at weekends he improved somewhat, though when he went on holiday he observed it took 3–4 days before he felt entirely free of symptoms. He had no previous history of asthma, hay fever or rhinitis. He smoked 10 cigarettes a day and was not known to have any drug allergy. He had one daughter with asthma.

There was no abnormality on physical examination.

Haemoglobin, white cell count and differential were normal, as was his chest X-ray. Prick testing was positive with house dust, *Dermatophagoides farinae* and *D. pteronyssinus*, and negative with ampicillin and related substances.

His respiratory function tests on admission showed:

	Actual	% Predicted
FEV ₁	3600 ml	100
FVC	4900 ml	110
FEV ₁ /FVC%	74%	
VC	4600 ml	107
FRC	3500 ml	100
TLC	6900 ml	110
RV/TLC%	33.4%	
Airways resistance		
cm H ₂ O/l/sec	1.67	104
(Body plethysmograph at lung volume)	(3630 ml)	
CO gas transfer factor		
(single breath)	30.9	110

He was admitted to hospital on three separate occasions. (1) October 2–9, 1972; (2) December 12–21, 1972; and (3) January 8–13, 1973. The materials with which he was challenged, the amounts, the dates and the duration of the provocation tests are shown in Table I(a).

Patient 2, male, aged 26 years, began working in the extraction department at the age of 23 years. Initially he was mostly in contact with the dust of 6APA. After 4 months he developed an erythematous dry rash on his hands and face. This cleared spontaneously but occasionally recurred. Eighteen months later he moved to a different part of the factory with less exposure to penicillin dusts, but after a month developed an attack of wheezing. This occurred several hours after a temporary secondment to the 6APA production line. On returning to work 4 weeks later he developed a further attack 8 hr after entering the factory, following which he was transferred to the 'penicillin free' building. Seven days later he developed his third attack of wheezing, which he related to contact over the tea break with friends from the extraction department whose clothes were contaminated with penicillin dusts. He had no previous history of asthma, eczema, rhinitis or drug allergy. He had a daughter with eczema and his father was said to have had 'penicillin allergy'. He smoked 30 cigarettes a day.

There was no abnormality on physical examination.

Haemoglobin, white blood count and differential were normal, as was his chest X-ray and urinalysis. Skin testing was negative to the 23 inhalant and food allergens as well as to ampicillin and related substances.

Table 1a. Inhalation tests in patient 1

		Patient 1			
Materials		Amount of material added to 250 g lactose	Duration of challenge	Dates of challenge	Results maximum % fall from pre-challenge FEV ₁
Inhalation challenge					
Lactose		—	30 min	2.10.72	16
		—	30 min	11.10.72	13
		—	30 min	16.10.72	10
		—	1 hr	11.12.72	0
		—	1 hr	14.12.72	2
Ampicillin	Commercial	10 g	30 min	17.10.72	25
		10 g	30 min	12.12.72	23
		10 g	1 hr	19.12.72	46
	Purified	10 g	1 hr	8. 1.73	44
	Chemical	10 g	1 hr	12. 1.73	32
6APA	Commercial	10 g	1 hr	11. 1.73	16
	Purified	10 g	1 hr	10. 1.73	23
Benzyl penicillin	Commercial	—			
	Purified	—			
Benzyl penicilloyl polylysine		20 mg	1 hr	18.12.72	6
6APA protein impurities		—			
<i>E. coli</i> amidase extract		—			
Oral challenge	Amount given in two capsules				
Commercial ampicillin		500 mg		9. 1.73	14
Commercial benzyl penicillin		—			

His respiratory function tests on admission were:

	Actual	% Predicted
FEV ₁	4600 ml	100
FVC	6358 ml	114
FEV ₁ /FVC%	72.2%	
VC	5850 ml	104
FRC	4500 ml	106
TLC	8250 ml	106
RV/TLC%	29.2%	
Airways resistance		
cm H ₂ O/l/sec	1.1	
(Body plethysmograph at lung volume)	(4500 ml)	
CO gas transfer factor		
(single breath)	30.2	80

He was admitted to hospital on two separate occasions. (1) December 1–23, 1972; and (2) January 23 to February 3, 1973. The materials with which he was challenged, the amounts, the dates and the duration of the provocation tests are shown in Table 1(b).

Table 1b. Inhalation tests in patient 2

Patient 2					
Materials		Amount of material added to 250 g lactose	Duration of challenge	Dates of challenge	Results maximum % fall from pre-challenge FEV ₁
Inhalation challenge					
Lactose		—	30 min	5.12.72	9
		—	30 min	23. 1.73	3
Ampicillin	Commercial	1 g	30 min	12.12.72	36
	Purified	1 g	30 min	26. 1.73	16
	Chemical	1 g	30 min	24. 1.73	36
6APA	Commercial	1 g	30 min	6.12.72	44
	Purified	1 g	30 min	11.12.72	7
		1 g	30 min	25. 1.73	3
Benzyl penicillin	Commercial	1 g	30 min	31. 1.73	46*
	Purified	—			
Benzyl penicilloyl polylysine		20 mg	30 min	18.12.72	3
6APA protein impurities		20 mg	30 min	21.12.72	6
<i>E. coli</i> amidase extract		500 mg	30 min	14.12.72	4
Oral challenge		Amount given in two capsules			
Commercial ampicillin		500 mg		29. 1.73	36
Commercial benzyl penicillin		—			

* Accompanied by fall in CO gas transfer factor from 30.2 to 22.7.

Patient 3, male, aged 44 years, began employment in the production of penicillin antibiotics at the age of 42 years. He came into contact particularly with the fine dusts of benzyl penicillin and *E. coli* amidase preparation, rarely encountering the dusts of 6 amino penicillanic acid and ampicillin. After 2 years he developed conjunctivitis, and penicillin allergy was suspected. He wore a protective hood which led to the abatement of these symptoms. Two months later he developed eczema on his hands and, after a further month, rhinitis and asthma. He noted that the wheezing began 6–8 hr after starting work and resolved within 2 hr of returning home. The symptoms became more severe necessitating 3 days off work. He was transferred to the 'penicillin free' building and remained without symptoms for 1 month before developing further attacks of cough and wheeze. He had no previous history of asthma, rhinitis, eczema or drug allergy, but one of his two sons had asthma and his daughter, eczema. He smoked 10 cigarettes a day.

There was no abnormality on physical examination.

Haemoglobin, white blood count and differential were normal, as was his chest X-ray. Skin testing was negative to the 23 inhalant and food allergens as well as to ampicillin and related substances.

His respiratory function tests on admission were:

	Actual	% Predicted
FEV ₁	1950 ml	68
FVC	3770 ml	100
FEV ₁ /FVC%	52%	
CO gas transfer factor (single breath)	23	93

Table 1c. Inhalation tests in patient 3

Patient 3					
Materials		Amount of material added to 250 g lactose	Duration of challenge	Dates of challenge	Results maximum % fall from pre-challenge FEV ₁
Inhalation challenge					
Lactose		—	30 min	2. 2.73	21
Ampicillin	Commercial	1 g	30 min	15. 2.73	36
	Purified	1 g	30 min	16. 2.73	43
	Chemical	—			
6APA	Commercial	1 g	30 min	17. 2.73	14
		1 g	30 min	9. 2.73	19
	Purified	1 g	30 min	8. 2.73	17
Benzyl penicillin	Commercial	1 g	30 min	5. 2.73	65
	Purified	1 g	30 min	6. 2.73	48
Benzyl penicilloyl polylysine		—			
6APA protein impurities		—			
<i>E. coli</i> amidase extract		—			
Oral challenge		Amount given in two capsules			
Commercial ampicillin		—			
Commercial benzyl penicillin		500 mg		12. 2.73	59

He was admitted to hospital from February 1–18, 1973. The materials with which he was challenged, the amounts, the dates and the duration of the provocation tests are shown in Table 1(c).

Patient 4, male, aged 39 years, began work in the extraction department at the age of 27 years, where he came into contact with the dusts of benzyl penicillin and 6 amino penicillanic acid. Four years after starting work he developed shortness of breath on walking quickly on the flat, and a daily productive cough. He noticed that heavy exposure to dusts of any nature made his breathing worse. He had no history of attacks of wheezing, sneezing or eczema, and had no past history of drug allergy. He smoked 20 cigarettes a day. There was no family history of asthma, eczema or rhinitis.

On physical examination there was no abnormality.

Haemoglobin, white cell count and differential were normal as was his chest X-ray. Skin testing was negative to the 23 inhalant and food allergens as well as to ampicillin and related substances.

His respiratory function tests on admission were:

	Actual	% Predicted	After isoprenaline
FEV ₁	2600 ml	84	2700 ml
FVC	4092 ml	100	4100 ml
FEV ₁ /FVC%	63%		66%
CO gas transfer factor (single breath)	19.3	76	

Table 1d. Inhalation tests in patient 4

		Patient 4			
Materials		Amount of material added to 250 g lactose	Duration of challenge	Dates of challenge	Results maximum % fall from pre-challenge FEV ₁
Inhalation challenge					
Lactose		—	30 min	7.11.72	10
Ampicillin	Commercial	10 g	30 min	9.11.72	8
	Purified	—			
	Chemical	—			
6APA	Commercial	—			
	Purified	—			
Benzyl penicillin	Commercial	10 g	30 min	10.11.72	11
	Purified	—			
Benzyl penicilloyl polylysine		—			
6APA protein impurities		—			
<i>E. coli</i> amidase extract		—			
Oral challenge	Amount given in two capsules				
Commercial ampicillin		—			
Commercial benzyl penicillin		—			

He was admitted to hospital from November 5–10, 1972. The materials with which he was challenged, the amounts, the dates and the duration of the provocation tests are shown in Table 1(d).

Results

Control days

All patients had at least one control day when inhalation testing was carried out with the lactose vehicle alone, and these results are shown together with the date and duration of exposure to lactose in Table 1a, b, c and d. The results are expressed as the maximum percent fall from the FEV₁ prior to exposure to the lactose. During some of these control days, the FEV₁ fell by over 15%. Possible reasons for this decrease in FEV₁ were illustrated by patient 1. During the first control day only 3 days after factory exposure to ampicillin, his FEV₁ fell by 16%. The maximum fall occurred during the night, 16 hr after exposure to the control lactose vehicle. However, when he had been away from the factory for 2 months, his FEV₁ following a further control challenge with the lactose vehicle, fell at its maximum 16 hr later by only 2%. Similarly, following a positive asthmatic reaction to ampicillin, the FEV₁ on the following day fell substantially below that on previous control days. It was only on the second post-challenge day that the FEV₁ approximated control levels. This is illustrated in Fig. 1. Ideally, therefore, it seems preferable to investigate the patients some weeks after their last factory exposure, but this may not always be convenient or economically possible for the patient. Similarly, it was necessary with some of the patients exhibiting late asthmatic reactions to carry out provocation challenge testing only every other day.

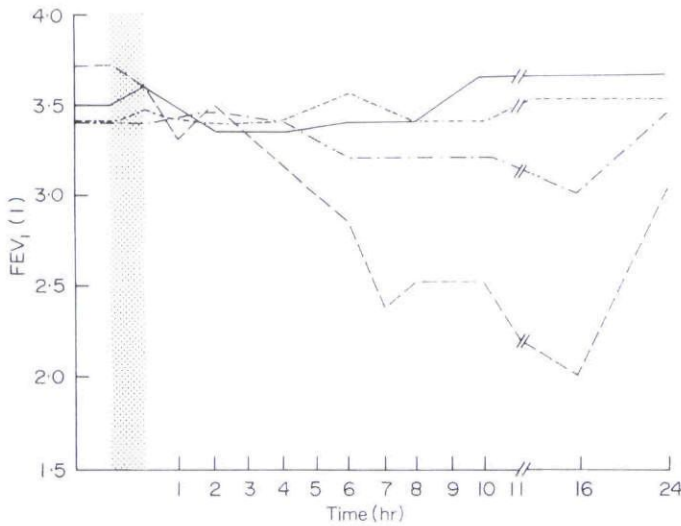


Fig. 1. Measurements of FEV_1 on days following a late asthmatic reaction to commercial ampicillin. Patient 1. —, 18.12.72, 250 g lactose; — —, 19.12.72, 250 g lactose + 10 g 'commercial' ampicillin; - · -, 20.12.72, no challenge; - · · -, 21.12.72, no challenge. Stippled area = challenge.

Pre-challenge FEV_1

The measurements of FEV_1 made on each pre-challenge occasion for the four patients are shown in Table 2. They are expressed as the mean values of the FEV_1 together with the range. The variation above and below the mean did not exceed 13%.

Provocation challenge tests

The results of these tests on the four patients are shown in Table 1a, b, c and d. They are presented as the maximum percent fall from the pre-challenge levels of FEV_1 . The material used in the provocation challenge, the amount, the date and the duration of the test are also tabulated. An asthmatic reaction was considered to occur when the FEV_1 fell by more than 15% of the pre-challenge level.

The induced asthmatic reactions were late in type; that is the airway obstruction developed slowly, beginning usually 1 hr or more after provocation challenge and was prolonged in nature with maximum falls in the FEV_1 occurring between 3 and 16 hr later.

FEV_1/FVC ratio

During the strong late asthmatic reactions the FEV_1/FVC ratio fell in patient 1 from

Table 2. FEV_1 range pre-challenge in the 4 patients

	Patient			
	1	2	3	4
Pre-challenge FEV_1				
Mean and	3.6	4.7	1.5	2.5
Range in litres	3.4-3.8	4.6-4.8	1.3-1.65	2.4-2.7

Table 3. Blood eosinophil counts before and after test

Patient	Mean eosinophil counts (mm ³)	
	Before positive asthmatic reaction	24 hr after positive asthmatic reaction
1	390	520
2	270	506
3	550	1042
4	(305)	(320)

74 to 61% December 19, 1972, in patient 2 from 72 to 55% January 31, 1973 and in patient 3 from 52 to 35% February 5, 1973.

Body temperature

In none of the patients during any of the provocation tests, did the oral temperature rise above normal.

Eosinophil and neutrophil polymorphonuclear leucocyte count

Patients 1, 2 and 3 developed blood eosinophilia which occurred within 24 hr of the first positive provocation challenge test. The counts tended to increase if positive asthmatic reactions occurred on subsequent days, and decrease in the absence of these reactions. No blood eosinophilia occurred in patient 4. The mean eosinophil counts for each patient before and 24 hr after approximately five positive asthmatic reactions are shown in Table 3. None of the patients developed an increase above normal in the neutrophil polymorphonuclear leucocytes. The counts for patient 4 are shown in brackets since he did not on any occasion develop late asthmatic reactions.

Patient 1 (Table 1a)

Inhalation challenge. Commercial ampicillin, ampicillin in its purified form and ampicillin (chemical) all gave rise to strong late asthmatic reactions. Provocation challenge with commercial 6APA and 6APA in its purified form produced only mild late falls in the FEV₁ of 16 and 23% respectively. These results are illustrated in Figs. 2 and 3.

Provocation challenge testing was carried out with benzyl penicilloyl polylysine. This substance is thought to be one of the major antigenic determinants of penicillin (Batchelor & Dewdney, 1968). No asthmatic reaction occurred with the 20 mg of this material which was all that could be tested.

Oral challenge. The taking by mouth of two capsules containing 500 mg of commercial ampicillin produced no asthmatic reaction (Table 1a).

Pharmacological agents. The effects of pharmacological agents on these reactions are shown in Table 4. The dates, the amount of material used and the duration of the challenge tests are similarly illustrated. Neither 40 mg of sodium cromoglycate, nor 200 µg of beclomethasone dipropionate administered 30 min before challenge blocked the late asthmatic reaction. Administration of 200 µg of beclomethasone dipropionate given both before and 3-hourly up to 12 hr after the challenge, blocked the late asthma, whilst 40 mg sodium cromoglycate, similarly given, had no definite inhibitory effect.

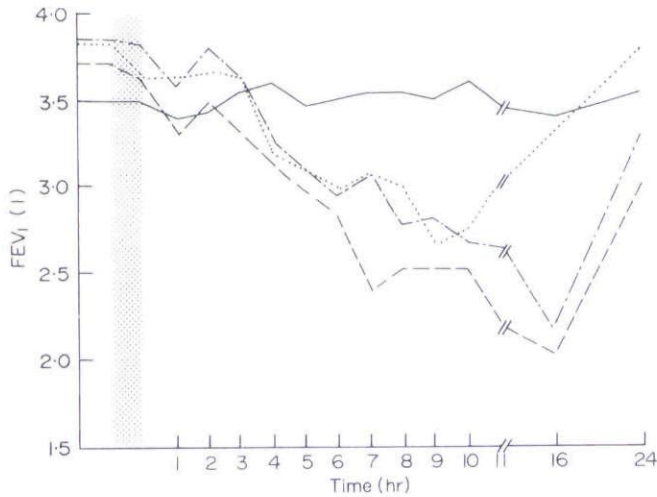


Fig. 2. Inhalation challenge tests—commercial, purified and chemical ampicillin. Patient 1. —, 14.12.72, 250 g lactose; ---, 19.12.72, 250 g lactose + 10 g 'commercial' ampicillin; - · -, 8.1.73, 250 g lactose + 10 g 'purified' ampicillin; ·····, 12.1.73, 250 g lactose + 10 g ampicillin (chemical). Stippled area = challenge.

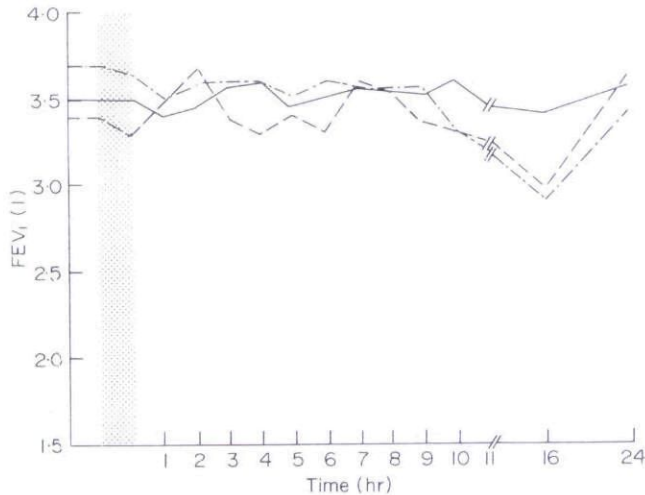


Fig. 3. Inhalation challenge tests—commercial and purified 6APA. Patient 1. —, 14.12.72, 250 g lactose; ---, 10.1.73, 250 g lactose + 10 g 'purified' 6APA; - · -, 11.1.73, 250 g lactose + 10 g 'commercial' 6APA. Stippled area = challenge.

Patient 2 (Table 1b)

Inhalation challenge. The results of inhalation and oral challenge testing with ampicillin and related substances are shown in Table 1b. Commercial ampicillin and ampicillin (chemical) gave rise to strong late asthmatic reactions, but this was not the case with purified commercial ampicillin, which produced a mild asthmatic reaction only. This is illustrated in Fig. 4. Similarly, although commercial 6APA induced a strong asthmatic reaction, none was produced by provocation challenge with the same amount in its purified 6APA form. This is illustrated in Fig. 5.

Table 4. Effect of sodium cromoglycate and beclomethasone dipropionate on inhalation test reactions.

Pharmacological agents	Dosage	Relation to provocation challenge	Patient 1				Patient 2			
			Amount of material added to 250 g lactose	Duration of challenge	Date	Maximum % fall from pre-challenge FEV ₁	Amount of material added to 250 g lactose	Duration of challenge	Date	Maximum % fall from pre-challenge FEV ₁
Lactose control										
Sodium cromoglycate	40 mg	30 min pre-challenge	1 g commercial ampicillin	30 min	16.10.73	10		30 min	5.12.72	9
Beclomethasone dipropionate	200 µg	30 min pre-challenge	1 g commercial ampicillin	30 min	7.10.72	31	1 g commercial 6APA	30 min	19.12.72	49
Sodium cromoglycate	40 mg	30 min pre- and 3-hourly post-challenge	1 g commercial ampicillin	30 min	6.10.72	29	1 g commercial ampicillin	30 min	7.12.72	0
Beclomethasone dipropionate	200 µg	30 min pre- and 3-hourly post-challenge	10 g commercial ampicillin	60 min	15.10.72	20	—			
Isoprenaline sulphate	0.16 mg	10 min pre-challenge	—	30 min	15.10.72	6		30 min	8.12.72	9
Isoprenaline sulphate	0.16 mg	1 hr post-challenge	—	30 min			1 g commercial 6APA	30 min	8.12.72	2
Placebo		30 min pre-challenge	1 g commercial ampicillin		12.10.72	40	1 g commercial 6APA	30 min	22.12.72	49

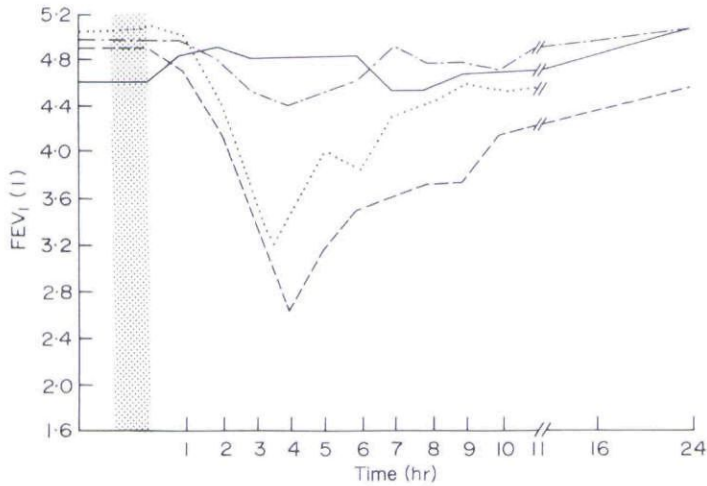


Fig. 4. Inhalation challenge tests—commercial purified and chemical ampicillin. Patient 2. —, 5.12.72, 250 g lactose; ---, 12.12.72, 250 g lactose + 1 g 'commercial' ampicillin;, 24.1.73, 250 g lactose + 1 g ampicillin (chemical); —·—, 26.1.73, 250 g lactose + 1 g 'purified' ampicillin. Stippled area = challenge.

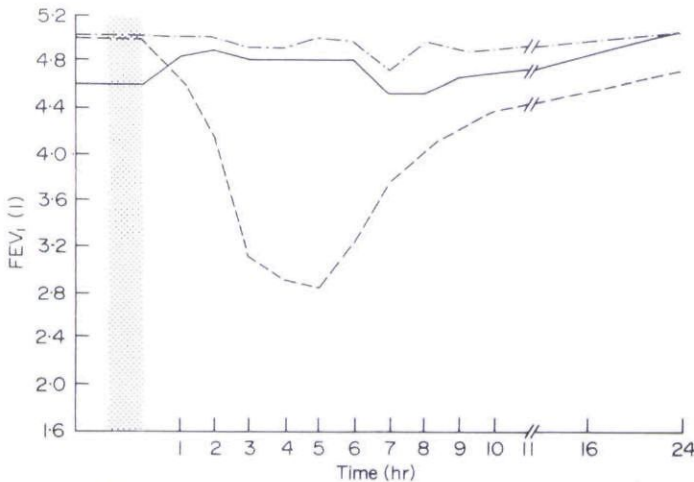


Fig. 5. Inhalation challenge tests—commercial and purified 6APA. Patient 2. —, 5.12.73, 250 g lactose; ---, 6.12.73, 250 g lactose + 1 g 'commercial' 6APA; —·—, 11.12.73, 250 g lactose + 1 g 'purified' 6APA. Stippled area = challenge.

The asthmatic reaction induced by inhalation challenge testing with 1 g of commercial benzyl penicillin in 250 g lactose was particularly prolonged. It was 4 days before the FEV_1 again approximated pre-challenge levels. Provocation testing was not carried out with 'purified' benzyl penicillin. Inhalation challenges tests with 6APA protein impurities benzyl penicilloyl polylysine and *E. coli* amidase extracts gave negative results.

The asthmatic reactions were paralleled by sneezing and rhinitis progressing to a blocked nose, and by a mild pruritic erythematous rash over the exposed skin of the face, hands and neck; the latter taking up to 48 hr to clear.

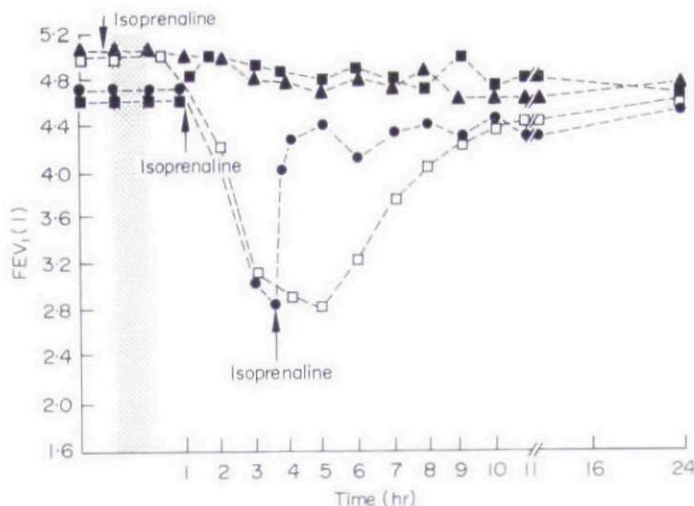


Fig. 6. Inhalation challenge tests—effect of isoprenaline. Patient 2. —□—, 6.12.73, 250 g lactose + 1 g 'commercial' 6 APA; —▲—, 8.12.73, isoprenaline 10 min before 250 g lactose + 1 g 'commercial' 6 APA; —●—, 15.12.73, isoprenaline 3½ hr after 250 g lactose + 1 g 'commercial' ampicillin; —■—, 20.12.73, isoprenaline 1 hr after 250 g lactose + 1 g 'commercial' ampicillin. Stippled area = challenge.

Oral challenge. The taking by mouth of two capsules containing 500 mg commercial ampicillin led to a late asthmatic reaction similar to that produced by inhalation of the same material. It was accompanied by gastrointestinal symptoms. One hour after ingesting the capsule of ampicillin, diarrhoea and colicky abdominal pains began, taking 10 hr to clear. This is illustrated in Fig. 9, and is summarized in Table 1b.

Pharmacological agents. The effects of pharmacological agents on these reactions are illustrated in Table 4. Inhalation challenge tests were carried out with 1 g of either commercial ampicillin or commercial 6APA in 250 g of lactose with the provocation challenge continuing for 30 min. 40 mg of sodium cromoglycate administered 30 min before the allergen failed to inhibit the asthmatic reaction, though this was blocked by similar pre-challenge inhalation of 200 µg of beclomethasone dipropionate. Of particular interest was the effect of inhalation of 0.16 mg of isoprenaline sulphate 10 min before provocation challenge, which inhibited the late asthmatic reaction. Administration of the same dose of isoprenaline on a separate occasion, 1 hr after allergen challenge, again blocked the expected asthmatic reaction. Further, the administration of 0.24 mg of isoprenaline sulphate during the late asthmatic reaction led to its reversal. This is illustrated in Fig. 6.

Patient 3 (Table 1c)

Inhalation challenge. The results of inhalation challenge testing with ampicillin and related substances are shown in Table 1c. Both ampicillin and benzyl penicillin, in commercial and purified forms, gave rise to late asthmatic reactions. Ampicillin (chemical) was not tested in this man. The reactions with benzyl penicillin are illustrated in Fig. 7. However, neither commercial nor 'purified' 6APA produced asthma on inhalation challenge, as is shown in Fig. 8.

Rhinitis occurred at the same time as the late asthmatic reactions and a mild

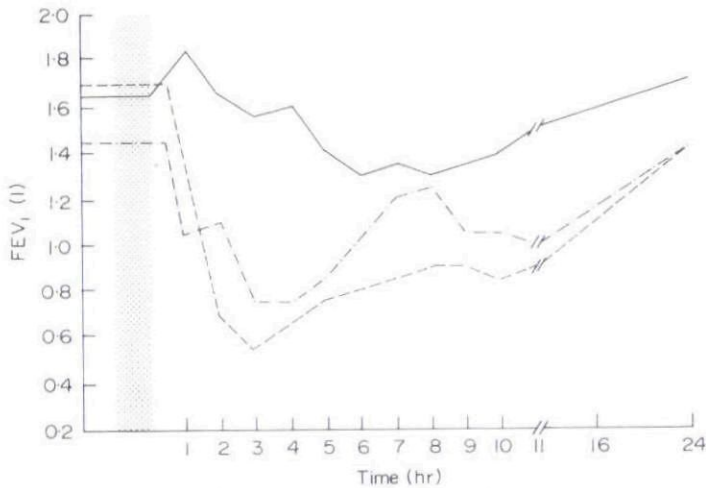


Fig. 7. Inhalation challenge tests—commercial and purified benzyl penicillin. Patient 3. —, 2.2.73, 250 g lactose; ---, 5.2.73, 250 g lactose + 1 g 'commercial' benzyl penicillin; - - -, 6.2.73, 250 g lactose + 1 g 'purified' benzyl penicillin. Stippled area = challenge.

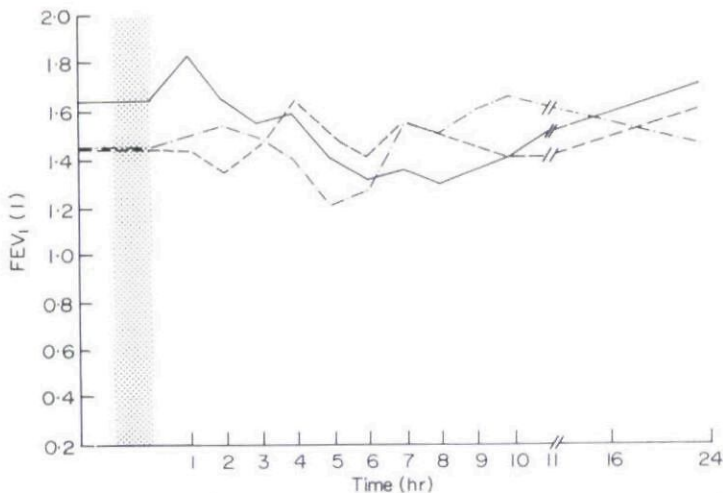


Fig. 8. Inhalation challenge tests—commercial and purified 6APA. Patient 3. —, 2.2.73, 250 g lactose; ---, 8.2.73, 250 g lactose + 1 g 'purified' 6APA; - - -, 17.2.73, 250 g lactose + 1 g 'commercial' 6APA. Stippled area = challenge.

pruritic erythematous rash developed over the exposed area of the face and neck taking up to 48 hr to resolve.

Oral challenge. The taking by mouth of two capsules containing 500 mg of benzyl penicillin produced a late asthmatic reaction beginning only 30 min after ingestion and lasting 24 hr. This was accompanied by extensive urticaria but not by diarrhoea. This reaction is illustrated in Fig. 9 and summarized in Table 1c.

Patient 4

Inhalation challenge. There was no evidence of significant reversible airways disease in this patient. The results of inhalation challenge testing are shown in Table 1d.

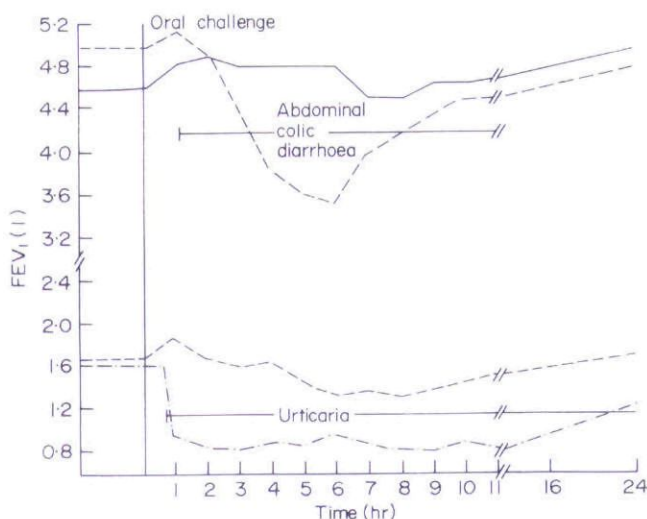


Fig. 9. Oral challenge test—commercial ampicillin and commercial benzyl penicillin. Patient 2. —, 5.12.72, control; —, 29.1.73, 500 mg capsules 'commercial' ampicillin. Patient 3. - - -, 2.2.73, control; —, 12.2.73, 500 mg capsules 'commercial' benzyl penicillin.

Inhalation challenge with 10 g of commercial ampicillin and 10 g of commercial benzyl penicillin for 30 min was followed by falls in FEV₁ of 8 and 11% only, comparable with the 10% on control testing with the lactose vehicle.

The results of provocation challenge tests in the four patients are summarized in Table 5.

Discussion

Penicillin antibiotics are well known as causes of allergic reactions during treatment and it is not surprising that some of those involved in their manufacture should also develop allergic phenomena. It is interesting to note in the three factory workers described here, having almost daily contact with the fine dusts of ampicillin and related substances, that it was 2 years before asthma began, though itchy skin rashes developed earlier.

One of the difficulties in the investigation of these patients was the variability of the FEV₁ on initial control days. Ideally, stable control measurements are preferable for confident assessment in this type of study, but it may be possible to obtain very suggestive results from patients who show variation in control measurements of respiratory function. It became apparent in this study that the longer the patients were away from factory exposure, the more stable were their control measurements of FEV₁.

Further, in some patients provocation testing led to small but definite falls in the FEV₁ on subsequent days, paralleling in time course the initial reaction. Asthmatic reactions lasting many days, even weeks, have been reported following a single exposure to certain industrial chemicals (Pepys *et al.*, 1972a). After exposure to the enzymes of *B. subtilis* these asthmatic reactions have shown marked periodicity recurring at similar times on subsequent days (Mitchell & Gandevia, 1971). For these reasons it is preferable in some patients to delay investigation for several weeks after their last occupational exposure and when necessary to allow an adequate time interval between provocation challenge tests for this recurring asthmatic reaction to disappear.

In the three workers developing asthma, of whom two were non-atopic and one

Table 5. Summary of inhalation test reactions

Late asthmatic reactions to inhalation and oral challenge									
Patients	Inhalation challenge				Oral challenge				
	Ampicillin		6APA		Benzyl penicillin		Commercial		
	Commercial	Chemical	Purified	Commercial	Purified	Commercial	Purified	Ampicillin	Benzyl penicillin
1	+	+	+	±	±			0	
2	+	+	±	+	0	+		+	
3	+		+	0	0	+	+		+
4	0					0			

+ = marked late asthmatic reactions; ± = weak late asthmatic reactions; 0 = no reaction.

atopic, the type of asthma resulting from provocation challenge was late in type being slow to develop but progressive and prolonged in nature. In none of the patients was skin testing positive to solutions of ampicillin and related substances. During the asthmatic reactions, however, blood eosinophilia developed. There was no rise in body temperature. These findings are similar to those described with other inhaled materials giving rise to late asthma, namely wood dusts (Pickering, Batten & Pepys, 1972; Gandevia & Milne, 1970), piperazine dihydrochloride (Pepys *et al.*, 1972c), tolylene diisocyanate (Pepys *et al.*, 1972a) and the complex salts of platinum (Pepys, Pickering and Hughes, 1972b).

It must be emphasized that the respiratory reactions in these patients were monitored solely by measurements of FEV₁, FVC and PEF. In cases of late asthma produced by the inhalation of extracts of Western Red Cedar, the measurements of maximum expiratory flow at 50% of vital capacity and 60% of total lung volume revealed the presence of immediate changes in airway function not detected by measurement of FEV₁ or airway resistance (Chan-Yeung, 1973). The use of such measurements in the investigation of the patients discussed in this paper might have provided further information perhaps revealing the presence of immediate asthma preceding the late reactions.

The three patients differed in respect to the substances which produced late asthmatic reactions. Patient 1 developed a strong late asthmatic reaction to commercial ampicillin and similar late asthmatic reactions to 'purified' ampicillin as well as ampicillin (chemical). On the other hand the reactions developing with commercial or 'purified' 6APA were mild.

The absence of any difference in the asthmatic reactions to commercial or 'purified' products or to ampicillin produced by a different method is evidence against impurities being important in the aetiology of these late asthmatic attacks. Ampicillin is manufactured from the 6APA 'nucleus' by the addition of a side chain. The very slight reactions to 6APA suggest that the important allergen for this patient seems to be in relation to the extra side chain linked to the 6APA nucleus.

Patient 2 developed late asthmatic reactions on exposure to commercial ampicillin and ampicillin (chemical) but only a very slight reaction to 'purified' ampicillin (a 16% fall in FEV₁). Similarly, though late asthma occurred on exposure to commercial 6APA, none followed exposure to 6APA in purified form. Commercial benzyl penicillin also provoked late asthma. It would appear that the major allergen for this patient is an impurity. 6APA is produced by most commercial processes from benzyl penicillin by the use of an *E. coli* amidase enzyme to split off the phenyl acetyl side chain. Protein impurities including remnants of *E. coli* enzymes have been suggested in the past as a possible cause for allergic reactions (Batchelor *et al.*, 1967; Stewart, 1967). However, in this patient, *E. coli* protein impurities are unlikely to be the cause of the allergic reaction, since late asthma was induced by exposure both to benzyl penicillin and ampicillin (chemical) manufactured by a chemical method not involving the use of *E. coli* amidase. The impurity in this case, it appears, is present at the benzyl penicillin stage. Provocation challenge with 'purified' benzyl penicillin would have been desirable, but this was not possible because of the large number of tests already made in this patient. Provocation challenge tests with 6APA protein impurities and *E. coli* amidase extracts were negative but these materials could only be obtained in small amounts, which may have been insufficient to induce asthmatic reactions.

Patient 3 developed late asthmatic reactions when exposed to ampicillin and benzyl penicillin whether commercial or purified, but did not show any reaction in inhalation challenge with commercial or purified 6APA. This would suggest that the important allergens for this patient, like patient 1 but unlike patient 2, are related to the structure of ampicillin and benzyl penicillin, rather than the impurities that they may contain. The main difference between 6APA and benzyl penicillin from which it is made, and ampicillin into which it is made, is that 6APA represents the nucleus of the penicillin to which the appropriate side chains are linked, giving the other products. The reasons why these materials behave differently in their ability to produce asthma in the patients is not clear.

Patient 4 failed to show any asthmatic reaction on challenge with commercial ampicillin or benzyl penicillin in amounts provoking asthma in the other patients. There was no reversibility in his mild airway obstruction, and this finding, together with his history, classifies him as having chronic obstructive bronchitis.

Inhalation challenge testing as described in this paper can be of considerable value in determining first whether asthmatic reactions are related to contact with industrial chemical agents and then in clarifying the complex problem of which chemicals are the clinically important allergens in individual patients.

In these patients who had developed asthma following inhalation of the dusts of ampicillin and related substances, it was clearly necessary for therapeutic reasons, to discover the effect of giving these antibiotics by mouth in clinical doses. Two of the three patients developed late asthma associated with gastrointestinal symptoms and signs in one, and urticaria in the other, showing that the important allergens readily cross the gastrointestinal tract. Asthma following the ingestion of a number of possible allergens including alcoholic beverages has been described, with in some cases immediate asthma being blocked by prior inhalation of sodium cromoglycate (Davies, 1970; Breslin, Hendrick & Pepys, 1973). In neither of the patients described was the reaction severe nor was any therapeutic intervention necessary for reversal. However, administration of more than 500 mg of ampicillin orally or the injection of benzyl penicillin intramuscularly may well have led to severe allergic reactions. These results imply that the therapeutic use of these antibiotics in these workers is contraindicated. Oral challenge testing carefully performed in hospital can therefore be useful in deciding whether or not a patient's suspected allergy to therapeutic agents is clinically important.

The late asthmatic reactions provoked by ampicillin and related substances resemble those produced on inhalation challenge with *Aspergillus fumigatus* (McCarthy & Pepys, 1972), and like them may be mediated by a type 3 allergic mechanism. These late asthmatic reactions were blocked by the inhalation of 200 µg of beclomethasone dipropionate given in one case before and in the other, before and 3-hourly after the provocation test.

These results are compatible with the hypothesis that the late reactions are mediated by immune complex disease (Pepys *et al.*, 1974). Inhalation of 40 mg of sodium cromoglycate before in one case, and before and 3-hourly after in the other, failed to inhibit these reactions, though similar late reactions to other chemical agents such as piperazine have been inhibited by sodium cromoglycate given prior to and 3 hr after the provocation challenge (Pepys *et al.*, 1972c). It is thought that the inhibitory effect of sodium cromoglycate on late asthmatic reactions may be due to an inhibitory effect on immediate reactions which may serve to introduce the late reac-

tion. The dependence of type 3 allergic reactions on a preceding type 1 reaction is well established in animals (Cochrane, 1971) and in man (Pepys, 1969).

The results of administration of isoprenaline sulphate in patient 2 are of particular interest. 0.16 mg of isoprenaline sulphate inhaled either 10 min before or 1 hr after the provocation challenge inhibited the late asthma. Further 0.24 mg of isoprenaline sulphate inhaled during the actual asthmatic reaction led to its reversal. This effect of β adrenergic receptor stimulant drugs on late asthmatic reactions requires further study, since the reported action of these drugs administered during late asthma is that of only temporary and partial reversal of the airway obstruction (Pepys, 1973). β adrenergic receptor stimulant drugs act by stimulating adenylyl cyclase leading to an increase in cyclic AMP which inhibits histamine release (Robison, Butcher & Sutherland, 1967; Bourne & Melman, 1971). These agents can therefore block or modify type 1 allergic reaction. It may be that the inhibitory action of isoprenaline sulphate administered 1 hr after the provocation challenge is on the same basis. The rapid reversal of the late asthmatic reaction by inhalation of this drug could be attributed to its bronchodilator action, however that may be produced.

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