

Protection tests on bronchial allergen challenge with disodium cromoglycate and thiazinamium

H. Booij-Noord, N. G. M. Orie, W. Chr. Berg, and K. de Vries, Haren, Groningen, The Netherlands

The efficacy of the new antiallergic drug, disodium cromoglycate, has been studied with protection tests on bronchial allergen challenge in 17 patients with reversible obstructive lung disease. There was, in general, a markedly protective effect on the immediate allergic reaction without concomitant bronchodilatation. Thiazinamium also produced a very good protective effect, probably the result of the combination of bronchodilation and a specific antihistaminic activity.

Disodium cromoglycate (Intal, Lomudal) has been introduced as an anti-allergic drug. Altounyan¹ demonstrated the inhibition of a pollen antigen-induced asthmatic attack by a preceding inhalation of disodium cromoglycate. When this drug was given after the allergen challenge, there was almost no inhibitory effect. Therefore a prophylactic activity on an allergic, reaginic-type reaction, without causing bronchodilation, was postulated.

Pepys and associates² confirmed the result of Altounyan with disodium cromoglycate on the immediate reaction to extracts of grass pollen and *Dermatophagoides culinea* in 5 asthmatic patients, but Herxheimer and Bewersdorff³ found that protection with disodium cromoglycate was present in only 2 of 13 cases, and partial protection was possible in 3 cases. Several clinical trials seemed to confirm its therapeutic value to a certain degree,⁴⁻¹¹ although the combination of disodium cromoglycate with isoproterenol was often used and is not ideal for this type of investigation. Kidner and associates,¹² however, performed a double-blind trial without isoproterenol and demonstrated clinical improvement in 38 per cent of their patients.

From the Allergy Unit of the Pulmonary Division of Internal Medicine, University Hospital of Groningen, and Beatrixoord, Department of Chronic Obstructive Lung Diseases.

Supported by Grant GO 623 C21 from the National Research Council TNO, The Netherlands.

Received for publication Aug. 8, 1969.

Table I. *Clinical and laboratory data*

<i>Patient No.</i>	<i>Age (yrs.)</i>	<i>Sex</i>	<i>Attacks of dyspnea at rest</i>	<i>Chronic cough on rising or all day*</i>	<i>Chronic phlegm on rising or all day*</i>	<i>Vasomotor rhinitis or pollinosis</i>	<i>Corticosteroids (mg. prednisolone per day)</i>
1	32	F	+	+	+	+	-
2	15	F	+	-	-	-	7.5
3	13	M	+	+	+	-	-
4	14	F	+	-	-	+	10
5	21	M	+	-	-	+	-
6	23	M	+	-	-	-	-
7	34	F	+	+	-	+	-
8	14	M	+	-	-	+	-
9	17	F	+	+	-	-	-
10	15	M	+	-	-	+	-
11	15	M	+	+	+	-	-
12	21	M	+	+	+	+	-
13	35	M	+	-	-	+	-
14	18	M	+	-	-	+	-
15	15	M	+	+	+	+	-
16	64	M	+	+	+	-	-
17	12	M	+	+	+	-	5

*On most days of at least 3 months of the year, for at least 2 consecutive years.

†Measured early in the morning in the fasting condition before the first allergen inhalation.

‡Grade 0: no eosinophils seen on the slide; grade I: a few eosinophils, distributed among than 5 clusters of eosinophils on the slide; grade IV: exclusively, or almost exclusively, eosinophils

§Histamine aerosols in a spray with an airflow of 8 L. per minute containing exponentially measured by spirometry. The lowest concentration, causing a decrease in VC and/or FEV₁, of further decrease of at least 25 per cent. The concentrations used are: $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1 and 2, 4, 8, 16, for the reactivity of the bronchial tree. In normal subjects the histamine threshold value is over

As many patients with obstructive lung disease, especially young patients, have an evidently allergic component and as this component is often difficult to treat adequately, it seemed indicated to perform protection tests with disodium cromoglycate on allergen challenge.

In an earlier investigation¹³ thiazinamium (Multergan) produced a very good protective effect on allergen provocation, but the results were measured for only 12 minutes after the allergen inhalation, and the relationship between bronchodilation and the degree of protection was not analyzed. As many patients with reversible obstructive lung disease in our hospital are treated with this drug, protection tests with thiazinamium on allergen challenge were carried out in the same patients in order to be able to evaluate the efficacy of both drugs.

MATERIAL AND METHODS

Tests were performed on hospitalized patients with chronic obstructive lung disease with attacks of dyspnea, an allergic history, positive intracutaneous tests with one or more inhalant allergens, and nearly always blood eosinophilia. Before the allergen inhalation a control inhalation with the solvent of the allergen, coca solution, was performed for 10 minutes.

The first allergen inhalation test was conducted as a dose range finding. With house dust* the following doses were given: 1, 5, and 10 mg. per milliliter

*Prepared by Diephuis, Groningen; Wiesbadener Doppelinhalator, airflow 8 L. per minute.

Blood eosinophils (mm. ³)†	Sputum eosinophils (grades 0-IV)‡	Histamine threshold value (mg./ml.)§	Intracutaneous test with inhalant allergens			
			House dust (0.5 mg./ml.)	Molds (0.2 mg./ml.)	Pollen (1,000 NU)	Epidermals (0.25 mg./ml.)
495	III	4	+++	+	-	+++
860	III	1	++	++	-	++
375	III	1	++	+	++	+
1,100	III	32	++	+	-	++
640		16	++	+	+	++
540		4	+++	++	++	++
55	0	2	+++	++	-	++
1,110		2	+++	+++	+	+++
1,640	III		+++	-	-	+
560		8	+++	+	-	++
265		32	++	-	-	+
1,055	III	1	+++	++	+++	++
265	I-IV	8	++	-	-	-
475		32	+++	+	-	++
3,710	0-I	32	+++	-	-	+
1,100	0-II	8	-	++	-	-
100	III	4	++	++	-	-

neutrophils, on the slide; grade II: 1 to 5 clusters of eosinophils on the slide; grade III: more on the slide (magnification: 40×6.3).

increasing concentrations are inhaled during 30 seconds; the degree of bronchial obstruction is 10 per cent or over is called the threshold value, if this or the following concentrations give a and 32 mg. per milliliter (histamine diphosphate). This threshold value is considered to be an index 32 mg. per milliliter.

(weight lyophilized dry extract per volume coca solution); with molds*: 0.2 and 2 mg. per milliliter. Every dose was inhaled for 3 minutes, and vital capacity (VC) and forced expiratory volume in one second (FEV₁) were measured immediately afterward and 5 minutes and 10 minutes later.† If the highest dose did not produce a significant fall of FEV₁, then the allergen inhalation was continued for 10 minutes. After an effective dose the spirographic measurements were continued for 60 minutes.

Patients with stable results of VC and FEV₁, without reacting to coca inhalation but reacting to an allergen inhalation with a fall of FEV₁ of at least 600 ml., were submitted to the following program:

Test 1: first blank test

The dose producing a decrease of FEV₁ of 600 ml. or more in the dose range-finding test was inhaled.

Test 2: protection test with disodium cromoglycate

Disodium cromoglycate aerosol, a one per cent solution dissolved in distilled water, was given for 3 minutes (about 6 mg. disodium cromoglycate) 10 minutes before the allergen challenge, as described in Test 1.

*Prepared by Diephuis, Groningen; Wiesbadener Doppelinhalator, airflow 8 L. per minute.

†Lode spirometer D 53.

Table II. *Mean value and range of lung function data*

<i>Lung function tests</i>	<i>Number of investigated patients</i>	<i>Thiazinamium (25 mg. I.M.)</i>	
		<i>Before</i>	<i>After</i>
TLC (% predicted)	16	109 (85-136)	
RV as % of TLC	16	30 (17-42)	
VC (% predicted)	17	95 (68-118)	102 (75-121)
FEV ₁ (% predicted)	17	72 (30-95)	97 (41-120)
FEV ₁ as % of VC	17	58 (35-85)	73 (44-96)
FIV ₁ as % of VC	16	78 (56-94)	
Viscous work of breathing at rest (g.cm./ml.)	15	4.5 (3.0-12.0)	2.2 (0.6-4.5)

Test 3: protection test with thiazinamium

The same allergen challenge (see Test 1) was given 45 minutes after intramuscular administration of 25 mg. of thiazinamium.

Test 4: second blank test

The same allergen challenge was given as that in Test 1.

These allergen inhalations were always performed at the same time of the day; the interval between the tests was at least one day. The patients did not receive drugs 12 hours before the tests, except corticosteroids were given to 3 patients. Seventeen patients received these four tests, 15 with house dust inhalation and 2 with molds inhalation.

Some clinical data concerning these patients are shown in Table I; mean lung function data are given in Table II. Analysis of variance was used for comparing the results after the allergen inhalation in the four tests. Spearman's rank correlation was used for calculating the relationship between the degree of bronchodilation and the protective effect of the thiazinamium and the relationship between initial values of VC and FEV₁ and the degree of fall of VC and FEV₁, after the blank allergen inhalation in Tests 1 and 4.

RESULTS

The mean results of the 17 patients are shown in Figs. 1 and 2. As can be seen, there was a more marked decrease of FEV₁ than of VC in per cent of initial values. The maximal fall of VC and FEV₁ always occurred between 5 and 20 minutes after the allergen challenge. Thereafter the VC and FEV₁ increased spontaneously, although the VC and FEV₁ almost never reached the initial value within a period of 60 minutes. The lowest FEV₁ values after allergen inhalation in the four tests obtained in the individual patients are given in Fig. 3.

Comparison of the two blank tests

Statistically, there was no difference in the response on allergen challenge between the two blank tests ($p > 0.05$). In some persons (e.g., Patients 7, 10, 12, and 15) there was variability in degree of bronchial obstruction in Tests 1 and 4 but not in the same direction (Fig. 3). There was no correlation between

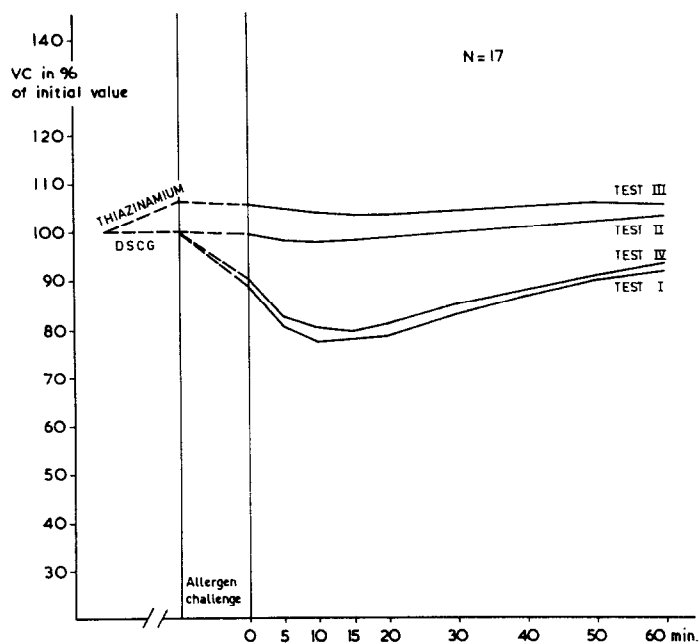


Fig. 1

Mean results of protective effect of disodium cromoglycate and thiazinamium measured with VC in the four tests.

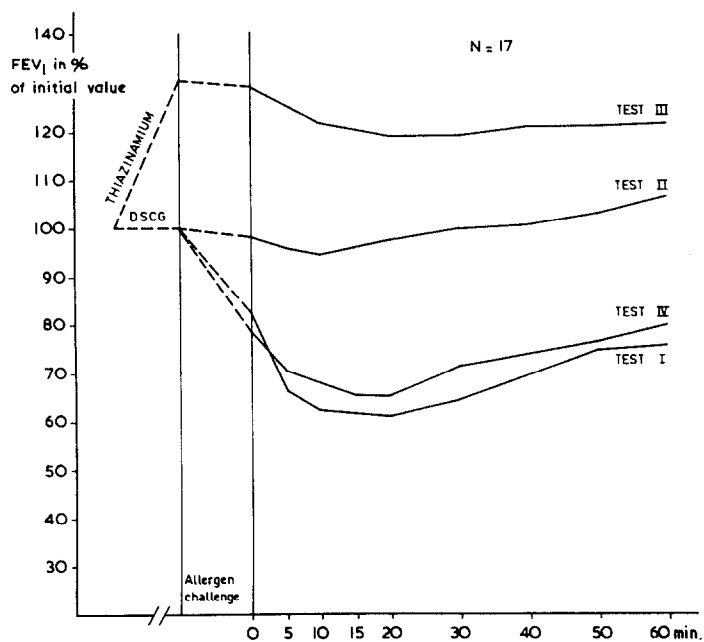
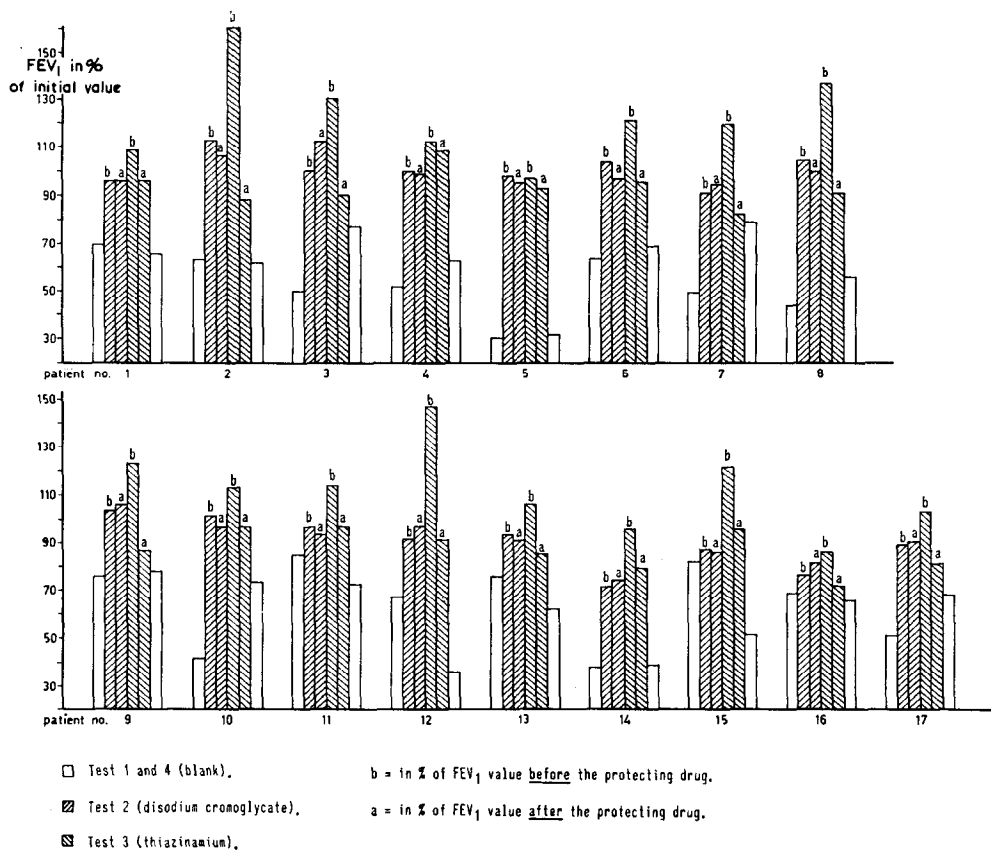


Fig. 2

Mean results of protective effect of disodium cromoglycate and thiazinamium measured with FEV₁ in the four tests.

**Fig. 3**

Minimal FEV₁ of individual patients after allergen inhalation in the four tests.

Table III. Comparison of the two blank allergen provocations

Patient No.	Difference between Tests 1 and 4 (4 to 1)			
	Initial value		Maximal decrease after allergen inhalation in % of initial value	
	VC (ml.)	FEV ₁ (ml.)	VC	FEV ₁
1	- 15	- 80	+ 4.7	+ 4.0
2	+ 40	- 42	- 7.1	+ 1.5
3	+417	+272	-24.0	-27.4
4	- 45	+137	- 3.6	-12.0
5	-161	- 10	+ 2.6	- 2.2
6	+790	+475	+ 3.3	- 6.4
7	- 22	-182	-22.5	-30.6
8	- 61	+153	- 4.8	-12.9
9	-126	-870	+11.9	- 2.2
10	+ 32	+105	-34.7	-42.2
11	- 22	0	+ 9.5	+12.7
12	+264	+325	+28.2	+30.3
13	-172	-103	+ 3.4	+14.3
14	+234	-122	+ 3.9	- 2.2
15	- 80	-859	+20.6	+29.9
16	+ 70	+150	- 2.5	+ 2.8
17	-400	-119	-13.6	-16.9

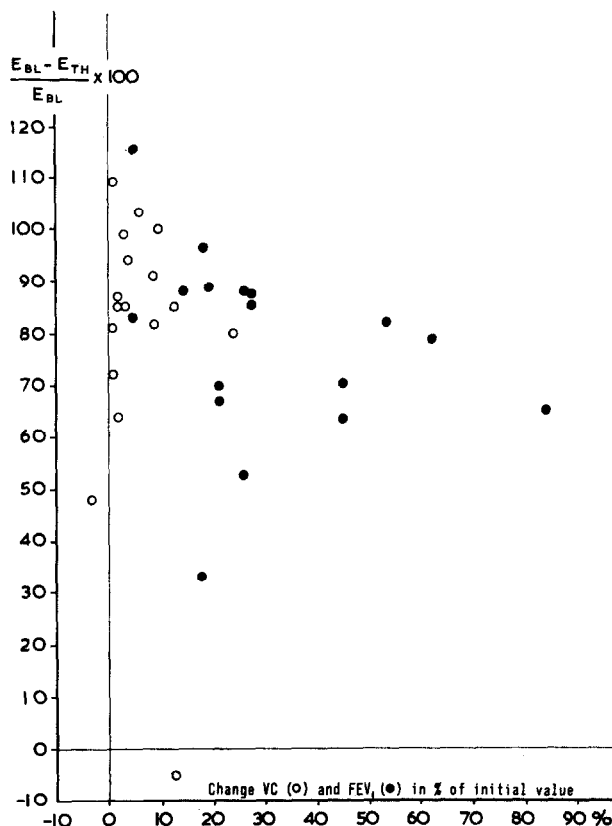


Fig. 4

Comparison of change of VC and FEV_1 with protective effect on allergen challenge after thiazinamium, 25 mg. intramuscularly E_{BL} = mean maximum fall VC and FEV_1 (milliliter) after allergen inhalation in the two blank Tests (1 and 4). E_{TH} = maximum fall VC and FEV_1 (milliliter) after allergen inhalation with thiazinamium (Test 3).

the initial value and the maximal fall of VC and FEV_1 following the allergen inhalation in Tests 1 and 4 with individual patients ($p > 0.05$, Table III).

Protective effect of disodium cromoglycate

In comparison with both blank tests an evident protection resulting from disodium cromoglycate could be demonstrated ($p < 0.005$). No bronchodilation or bronchial obstruction was apparent immediately and at 5 and 10 minutes following the inhalation of the drug.

Protective effect of thiazinamium

In comparison with both blank tests, thiazinamium gave good protection ($p < 0.005$). As could be anticipated, there was, in general, a bronchodilating effect with this drug: mean increase in VC 6 per cent (range 3 to 24 per cent); mean increase in FEV_1 30 per cent (range 4 to 84 per cent). No correlation was found between the increase of VC and FEV_1 and the protecting action ($p > 0.05$, Fig. 4).

**Protective effect of disodium cromoglycate
in comparison with that of thiazinamium**

In relation to the VC and FEV₁ measured after the administration of the drugs and immediately before the allergen inhalation, the protective effect of disodium cromoglycate was greater than that of thiazinamium ($p < 0.005$) as calculated from measurements 60 minutes after the allergen challenge. This could be the result of the falloff in the bronchodilator effect of thiazinamium during this period. We therefore compared the protective effect of both drugs during the first 20 minutes after the allergen inhalation, as the maximal fall of VC and FEV₁ always occurred in this time. No difference in the protective effects of thiazinamium and disodium cromoglycate was found in these circumstances ($p > 0.05$).

DISCUSSION

The results of this investigation clearly demonstrate the protective activity of disodium cromoglycate, confirming the findings of Altounyan¹ and Pepys and associates.² Thiazinamium also produced a pronounced protective effect on allergen challenge, a confirmation of an earlier investigation.¹³ In one patient only (No. 16, age 64) there was no appreciable protective effect with disodium cromoglycate, and in this individual thiazinamium produced the same insignificant degree of protection, although some bronchodilation could be demonstrated. It is remarkable that Herxheimer and Bewersdorff³ could establish protective effects with disodium cromoglycate only in some of their 13 investigated patients. As their experimental procedure was different from ours, we cannot explain the discrepancy in the results.

The two control tests were performed to ascertain reasonable quantitative reproducibility in order to validly demonstrate the protective activity of the drugs. The second blank test remained positive in all the patients, but in some cases there was a marked difference between the two blank tests. The bronchial obstruction after the allergen challenge was sometimes greater and sometimes less in the second blank test. In the literature¹⁴⁻¹⁶ two allergen inhalations are in general reported to give comparable results, although with some exceptions, and information is not always given as to the interval between the tests. There is no good explanation for the differences between the two blank tests, but some causes of spontaneous changes can be easily visualized. A relationship between the initial value of the VC and FEV₁ and its decrease after allergen inhalation could not be demonstrated. Hyposensitization with an amount of allergen which causes a mild reaction and hypersensitization with an amount of allergen which causes a sharp reaction, as mentioned by Herxheimer,¹⁷ also cannot explain the differences with the two blank tests in our cases.

Because the mechanisms of the immediate allergic reaction in the human lung (e.g., the responsible immunoglobulins, the participating cellular elements, and the active chemical mediators) have not been clearly defined, it is not easy to explain the mode of action of protective drugs. Cox¹⁸ reported that disodium cromoglycate has no anti-inflammatory properties and no bronchodilating activity, that no antagonizing action against histamine, SRS-A, bradykinin, or

serotonin could be demonstrated, and that no inhibition of the antigen-antibody union occurs. He suggested on the basis of experiments with animals and chopped human lung, passively sensitized with human reaginic serum, that the antiallergic activity was due to the prevention of disruption of mast cells resulting in inhibition of release of mediators, e.g., histamine and SRS-A. Orange and Austen^{19, 20} reported that pretreatment of rats with disodium cromoglycate inhibited the homocytotropic antigen-mediated release of histamine from mast cells but not the immunologic release of SRS-A^{rat} by polymorphonuclear leukocytes. The knowledge gained from studies in animals is often, however, not pertinent to human beings. Therefore an exact explanation of the protective effect of disodium cromoglycate is not possible at this moment.

Thiazinamium has antihistaminic and anticholinergic activities with bronchodilating effects. Anticholinergic drugs have, in general, no significant protective effects on the immediate reaction to allergen inhalation.^{13, 21} Only in some cases has a clear protective effect been reported, possibly the result of bronchodilation; a correlation between bronchodilation and protective effect was not investigated, however.^{13, 21} Antihistamines are specific antagonists of one of the likely contributing chemical mediators, histamine. In man they have mild but significant protective effects on allergen inhalation.^{13, 23} Moreover, bronchodilators (e.g., epinephrine, isoproterenol, and theophylline) also induce a protective effect on the immediate reaction of allergen provocation.^{13, 22}

There was no correlation between the bronchodilating effect of thiazinamium, measured as changes in VC and FEV₁, and the allergen protective effect in the present investigation. The protective effect of thiazinamium measured 60 minutes after the allergen inhalation was less pronounced than that of disodium cromoglycate in relation to the VC and FEV₁ measured after the administration of the drug and immediately before the allergen inhalation; 20 minutes after the allergen inhalation, however, there was no difference between the two drugs. This could be the result of the decrease of the bronchodilating effect of thiazinamium during the second hour after intramuscular administration, as was also demonstrated by Minette and Patigny.²⁴ Thiazinamium showed a more pronounced protective effect than the nearly pure antihistamine, mepyramine.¹³ At present we therefore suggest that the protective effect of thiazinamium was the result of a combination of specific antihistaminic and nonspecific bronchodilating effects, although no exact evidence is available. Actually, protection tests may furnish information not only about antiallergic drugs, but they also may provide clues about the basic mechanisms of the allergic reaction in the bronchial airways.

Is it possible to draw conclusions from this investigation for clinical application? In most reports about clinical trials with a single- or double-blind technique, the subjective improvement with disodium cromoglycate appears to be more dramatic than changes in objective criteria (e.g., FEV₁), although Robertson and associates²⁵ established improvement of other lung functions in some of their 11 investigated patients.

The present study gives objective data, but the clinical consequences are still uncertain. In the first place, besides an immediate reaction after allergen chal-

lenge, there also is a late bronchial reaction in many patients.^{16, 26} This late reaction starts 4 to 12 hours after allergen challenge and is often prolonged, sometimes lasting several hours. Since this type of reaction may be of great practical importance, it is therefore necessary to assess these late bronchial obstructive reactions in protection tests with disodium cromoglycate and thiazinamium. Moreover, many factors, endogenous, exogenous, and partly unknown, may play a role in patients with obstructive lung disease. Although the allergic component can be demonstrated, it is difficult to evaluate this component quantitatively, as is often the case with the other factors. It is therefore often impossible to predict the exact clinical results at this moment.

We thank Dr. R. E. C. Altounyan for detailed information and Fisons Ltd. for providing disodium cromoglycate. We also wish to thank Miss H. J. Buys, Miss M. G. E. v. d. Ham, Miss L. J. Muller, Miss H. J. Posthumus, Miss J. Drok and Miss B. Feenstra for technical assistance.

REFERENCES

1. Altounyan, R. E. C.: Inhibition of experimental asthma by a new compound—disodium cromoglycate "Intal," *Acta Allerg.* **22**: 485, 1967.
2. Peypys, J., Hargreave, F. E., Chan, M., and McCarthy, D. S.: Inhibitory effects of disodium cromoglycate on allergen-inhalation tests, *Lancet* **2**: 134, 1968.
3. Herxheimer, H., and Bewersdorff, H.: Disodium cromoglycate in the prevention of induced asthma, *Brit. Med. J.* **2**: 220, 1969.
4. Howell, J. B. L., and Altounyan, R. E. C.: A double-blind trial of disodium cromoglycate in the treatment of allergic bronchial asthma, *Lancet* **2**: 539, 1967.
5. Grant, I. W. B., Channell, S., and Drever, J. G.: Disodium cromoglycate in asthma, *Lancet* **2**: 673, 1967. (Letter to the Editor.)
6. Kennedy, M. C. S.: Disodium cromoglycate in asthma, *Lancet* **2**: 838, 1967. (Letter to the Editor.)
7. Lopez, M., Franklin, W., and Lowell, F. C.: A double-blind study of disodium cromoglycate in bronchial asthma, *J. ALLERG.* **41**: 89, 1968. (Abst.)
8. Morrison Smith, J., and Devey, G. F.: Clinical trial of disodium cromoglycate in treatment of asthma in children, *Brit. Med. J.* **1**: 340, 1968.
9. Moran, F., Bankier, J. D. H., and Boyd, G.: Disodium cromoglycate in the treatment of allergic bronchial asthma, *Lancet* **2**: 137, 1968.
10. Bruse, R. A., and Hansell, J. L.: Disodium cromoglycate in asthma, *Practitioner* **201**: 915, 1968.
11. Chen, J. L., Moore, N., Norman, P. S., and Van Metre, T. E., Jr.: Disodium cromoglycate, a new compound for the prevention of exacerbations of asthma, *J. ALLERG.* **43**: 89, 1969.
12. Kidner, P. H., Meisner, P., Pride, N. B., and Pearson, R. S. B.: Disodium cromoglycate in the treatment of bronchial asthma, *Lancet* **2**: 655, 1968.
13. Booij-Noord, H., Orie, N. G. M., ten Cate, H. J., Sloots, S., and Bolt, D.: The influence of various drugs on the vital capacity of asthmatics, *Int. Arch. Allerg.* **10**: 321, 1957.
14. ten Cate, H. J.: Onderzoek bij astmapatienten naar overgevoeligheid voor verstoven allergeenextracten, Thesis, Groningen, 1954.
15. Michgelsen, H. W. B.: Uitkomsten van het provocatie-onderzoek met extracten van inhalatie-allergenen bij patienten met astma en chronische bronchitis. Inhalatietest en inhalatietherapie. Verslag van een vergadering van de Belgische en Nederlandse Verenigingen voor Allergie, Antwerp, Jan. 17, 1959.
16. Citron, K. M.: The uses of bronchial sensitivity tests, *Acta Allerg. (Suppl. 8)* **22**: 17, 1967.
17. Herxheimer, H.: Bronchial hypersensitization and hyposensitization in man, *Int. Arch. Allerg.* **2**: 40, 1951.
18. Cox, J. S. G.: Disodium cromoglycate (FPL 670) ("Intal"): A specific inhibitor of reaginic antibody-antigen mechanisms, *Nature* **216**: 1328, 1967.
19. Orange, R. P., and Austen, K. F.: Guest editorial—Prospects in asthma therapy: Disodium cromoglycate and diethylcarbazine, *New Eng. J. Med.* **279**: 1055, 1968.
20. Orange, R. P., and Austen, K. F.: Pharmacologic dissociation of immunologic release of histamine and slow reacting substance of anaphylaxis in rats (33437), *Proc. Soc. Exp. Biol. Med.* **129**: 836, 1968.
21. Itkin, I. H., and Anand, S. C.: The role of atropine as a mediator blocker of induced bronchial obstruction, *J. ALLERG.* **41**: 88, 1968. (Abst.)

22. Herschfus, J. A., Rubitsky, H. J., Beakey, J. F., Bresnick, E., Levinson, L., and Segal, M. S.: Evaluation of therapeutic substances employed for the relief of bronchial asthma. A review, *Int. Arch. Allerg.* 2: 97, 1951.
23. Herxheimer, H.: Antihistamines in bronchial asthma, *Brit. Med. J.* 2: 901, 1949.
24. Minette, A., and Patigny, J.: Vergelijking van verschillende bronchodilatoren. Kritische studie van orciprenaline: Klassieke aerosolen en dosis-aerosol, *Tijdschr. Inst. Mijnhygiëne* 19: 3, 1964.
25. Robertson, D. G., Epstein, S. W., Warrell, D. A.: Trial of disodium cromoglycate in bronchial asthma, *Brit. Med. J.* 1: 552, 1969.
26. van Lookeren Campagne, J. G., Knol, K., and de Vries, K.: House dust provocation in children, *Scand. J. Resp. Dis.* 50: 76, 1969.