

Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone

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The immediate and late obstructive reactions after house dust inhalation were studied in 10 patients with reversible obstructive lung disease. The immediate obstructive reactions were of the conventional type. The late obstructive reactions occurred 4 to 8 hours after the allergen challenge and in this respect followed the time table of the Arthus reaction but were otherwise clearly distinct from the conventional Arthus reaction: marked bronchial obstruction, no crepitations, no fever, and no leukocytosis. Disodium cromoglycate showed an evident protective effect on the immediate reactions in contrast to prednisolone, which produced no protection in this type of reaction. There was a marked inhibitory effect on the late obstructive reaction with prednisolone and with disodium cromoglycate.

Patients in The Netherlands with chronic obstructive lung disease and an allergic component are mostly sensitive to house dust. Investigations of the immediate obstructive reaction occurring maximally 10 to 20 minutes after provocation have been performed earlier in combination with protection tests with different drugs.^{1, 2} The immediate obstructive reaction disappears within one to 3 hours, to be followed in many cases by a late obstructive reaction appearing 4 to 8 hours after inhalation and lasting several hours. Clinical details concerning this late reaction have been published,³⁻⁷ but lung function data are scarce; van Lookeren Campagne and associates⁸ studied house dust provocation in children, and the immediate and the late reaction were assessed by spirometry. There are some similarities between this late bronchial obstructive reaction and the late reaction after provocation with *Aspergillus fumigatus*, which has been regarded as a type III hypersensitivity reaction, as defined by Gell and Coombs.⁹

It was the goal of this investigation to examine in more detail the reactions that occur after house dust inhalation. The protective effects on the immediate

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and the late obstructive reactions of disodium cromoglycate and prednisolone were studied. This protective effect is measured for therapeutic purposes as well as for fundamental analysis of the reactions.

PATIENTS AND METHODS

Hospitalized patients with chronic obstructive lung disease were investigated while in a steady state and without bronchial infection. The subjects selected for study had attacks of dyspnea, a history of provocation by an allergen, positive intracutaneous test with one or more inhalant allergens, and nearly always blood eosinophilia.

Lung functions before and after challenge were recorded spirographically,* and the vital capacity (VC) and forced expiratory volume in one second (FEV₁) were noted. The test period lasted from 8 A.M. until 10 P.M. The VC and FEV₁ were at first determined 10 minutes, 5 minutes, and immediately before the house dust inhalation to obtain a base line (initial value). A solution of house dust,† 10 mg. per milliliter (dry weight dialyzed and lyophilized extract per milliliter Coca's solution), was nebulized‡ and inhaled for one minute. The VC and FEV₁ were measured immediately afterwards and 5 minutes later. When no reaction occurred, the inhalation was repeated for 4 minutes and eventually for 5 more minutes, with a 5 minute interval. If there was a fall of FEV₁ of 15 per cent or more of the initial value, then the VC and FEV₁ were also determined at 10, 15, 20, 30, 40, 50, and 60 minutes after the inhalation and thereafter every hour until 10 P.M. Sometimes during the late obstructive reaction, the FEV₁ decreased to such a low level that metaproterenol§ (Alupent) inhalation (5 per cent aerosol for 30 seconds) was indicated.

Design of experiment

Those patients who reacted with an immediate and a late obstructive reaction were submitted to the following program: Test 1: inhalation with Coca's solution; Test 2: first house dust inhalation; Test 3: protection tests with disodium cromoglycate (DSCG) (A two per cent solution dissolved in distilled water, aerosolized and inhaled for 3 minutes [approximately 12 mg.] 10 minutes before the allergen challenge); Test 4: second house dust inhalation (the same allergen challenge as in Test 2); Test 5: protection tests with prednisolone (orally 15 to 20 mg. daily for 9 to 16 days before and continued on the test day).

These five tests were performed in 10 patients. Relevant clinical data on the patients are shown in Table I, and lung function data are presented in Table II.¹⁰ The interval between the tests was at least 2 days. The patients did not receive medications for 12 hours before the tests, except prednisolone continuously in 2 patients (2.5 and 5 mg. daily). It had been shown previously that the dust extract employed did not produce bronchial obstruction in persons not allergic to house dust.⁸

Rectal temperature was taken at 11 A.M., 1 P.M., 3 P.M., and 5 P.M.; leukocytes were counted in the capillary blood at 8 A.M., 11 A.M., 1 P.M., 3 P.M., 5 P.M., and at 8 A.M. on the next day.

Intracutaneous tests with house dust were performed to assess direct and late skin reactions. Coca's solution was used as control. The following concentrations of house dust were used: 0.5, 0.05, 0.005, and 0.0005 mg. per milliliter. The injections were given at 9:40 A.M. and evaluated at 10 A.M. and thereafter at every hour till 10 P.M. in the evening.

Statistical analysis

Analysis of variance was used for comparing the results of spirometry after Coca's solution inhalation with house dust inhalation and with protection tests with DSCG and prednisolone.

*Lode Spirometer D 53, Lode Instruments, Groningen.

†Prepared by Pharmacy Diephuis, Groningen.

‡Wiesbadener Doppelinhalator, airflow (liters per minute).

§In Figs. 1 and 4, O stands for orciprenaline (metaproterenol).

TABLE IA. Clinical and laboratory data

Patient No.	Age (yr.)	Sex	Attacks of dyspnea at rest	Chronic cough on rising or all day*	Chronic phlegm on rising or all day*	Vasomotor rhinitis or pollinosis
1	18	F	+++	-	-	+
2	16	F	+	+	+	+
3	16	M	+++	-	-	+
4	19	F	++	-	-	-
5	15	M	+++	-	-	+
6	36	F	-	-	-	+
7	22	F	+++	-	-	-
8	21	F	+++	-	-	-
9	46	M	++	+	+	-
10	14	M	++	-	-	-

*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 (magnification: 40×6.3); Grade I: a few eosinophils, slide; Grade III: more than 5 clusters of eosinophils on the slide; Grade IV: exclusively, or

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

TABLE IB. Clinical and laboratory data

Patient No.	Age (yr.)	Sex	Intracutaneous tests						
			House dust (mg./ml.)				Molds (mg./ml.)		
			0.5	0.05	0.005	0.0005	0.2	0.02	0.002
1	18	F	++	+	-	-	+	-	-
2	16	F	+	+	+	-	++	+	-
3	16	M	++	+	-	-	+	-	-
4	19	F	+++	+	-	-	++	-	-
5	15	M	++	++	+	-	+	-	-
6	36	F	++	++	++	+	+	-	-
7	22	F	++	+	+	-	++	-	-
8	21	F	++	+	+	-	+	-	-
9	46	M	++	++	+	-	+	-	-
10	14	M	+++	++	-	-	+	-	-

||Mixed molds (5 pools of approximately 10 species) containing as main allergens: *Penicillium*

¶Pool of 8 grass pollens containing as main allergens: *Sceale cereale*, *Dactyles glomerata*, *Lolium*

#Series of 8 epidermals: horse, pig, cat, goat, cow, rabbit, dog, sheep.

RESULTS

The effects of house dust inhalation, with and without the effect of the inhibitor agents, are shown in Fig. 1 for an individual patient (No. 5). There was a marked, immediate obstructive reaction with a maximal fall of FEV₁ between 5 and 20 minutes after the inhalation. Spontaneous recovery and a return to normal of the spirometric values occurred 3 hours after the allergen inhalation. Thereafter the late reaction started which lasted several hours; at 5 P.M. the FEV₁ fell to 890 ml. and metaproterenol inhalations had to be administered.

The reaction after a second house dust inhalation was similar to that follow-

Wheezing		Blood eosinophils (mm. ³)†	Sputum eosinophils (Grades 0-IV)‡	Corticosteroids (mg. prednisolone per day)	Histamine threshold value (mg./ml.)§
At rest	With exertion				
-	+	760		-	8
-	+	715	III	-	4
+	+	850	III	-	16
-	+	470		-	2
+	+	300		-	32
+	+	240	III	2.5	1
+	+	430	III	-	4
+	+	200		-	2
+	+	330		-	2
+	+	560	II	5	1

distributed among neutrophils, on the slide; Grade II: 1 to 5 clusters of eosinophils on the almost exclusively, eosinophils on the slide.
increasing concentrations are inhaled during 30 seconds; the degree of bronchial obstruction is of 10 per cent or over is called the threshold value, if this or the following concentrations 2, 4, 6, 16, and 32 mg. per millimeter (histamine disphosphate). This threshold value is con-
threshold value is over 32 mg. per millimeter.

with inhalant allergens						
Pollen (NU)†			Epidermals (mg./ml.)#			
1,000	100	10	0.25	0.025	0.0025	0.00025
++	-		++	-	-	
+	-		++	+	-	
++	+	-	+	-	-	
+++	-		++	-		
+	-		++	+	-	
+++	+	-	++	++	++	+
+	-		+	+	-	
-			+			
-			++	+	+	-
-			+	-		

spp., Cladosporium spp., Aspergillus spp., and Altenaria spp.
perenne.

ing the first house dust inhalation. With disodium cromoglycate the immediate as well as the late obstructive reaction was completely prevented, whereas with prednisolone there was complete protection from the late obstructive reaction but not to the immediate reaction.

The mean results of the 10 patients, with and without protection are shown in Figs. 2 and 3. Results are expressed in per cent of initial value of VC and FEV₁.

Protective effect of disodium cromoglycate

These results are given as the mean values at the same time intervals after the allergen inhalation in the 10 patients. Disodium cromoglycate demonstrated

TABLE II. Lung function data

Patient No.	Age (yr.)	TLC (% predicted)	RV/TLC (%)	VC (ml.)	VC after thiazinamium*
1	18	125	26	3,140 (91)†	3,400
2	16	85	32	3,500 (87)	3,370
3	16	74	24	3,550 (68)	3,820
4	19	111	29	3,750 (112)	3,960
5	15	112	32	3,080 (97)	3,050
6	36	96	45	2,500 (68)	2,700
7	22	107	33	3,820 (98)	3,810
8	21	106	21	4,260 (99)	4,150
9	46	87	34	4,500 (100)	4,480
10	14	81	19	3,400 (70)	3,700

*Thiazinamium (Multergan) has antihistaminic and anticholinergic activities. It is used for assessing

†The numbers in parentheses are percentages of the predicted value.

‡The numbers in parentheses are percentages of VC.

§ND = not done.

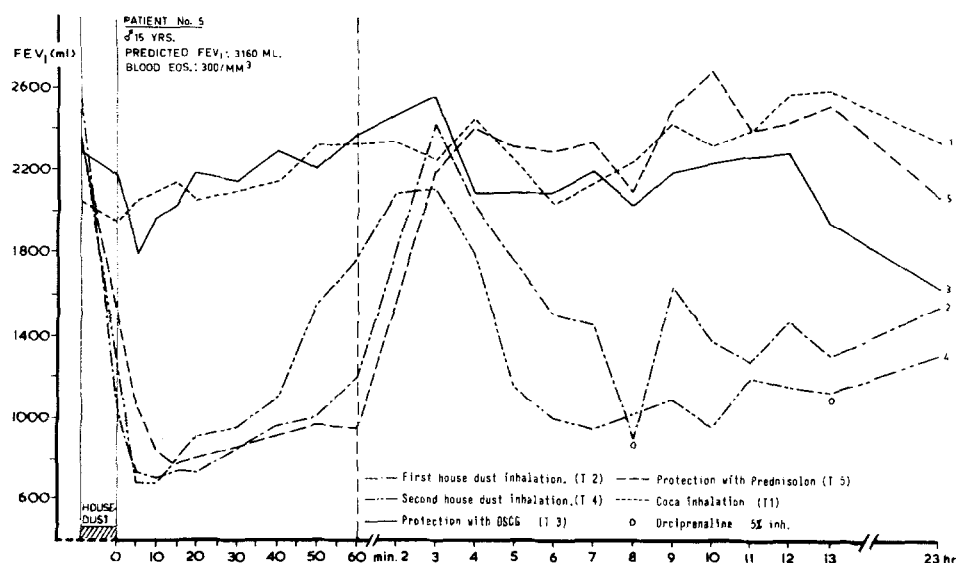


FIG. 1. The effect of DSCG and prednisolone on the immediate and late obstructive reactions after house dust inhalation in Patient No. 5.

an evident protection from the immediate ($p < 0.01$) and the late obstructive reactions ($p < 0.05$) after house dust inhalation.

The maximal fall of FEV₁ did not occur at the same time in all the patients, however, and there was also some difference between the first and the second house dust inhalations in the same patient. Accordingly, Fig. 4 illustrates for each individual patient the maximal fall of the FEV₁ in per cent of initial value in comparison with Coca's solution inhalation. In some patients with the inhalation of Coca's solution there was a spontaneous increase of FEV₁ starting at the end of the morning or in the afternoon, which explains the percentage of fall after house dust exceeding 100 per cent in Patient No. 3.

FEV ₁ (ml.)	FEV ₁ after thiazinamium	Viscous work of breathing ¹⁰ at rest (Gm. cm./ml.)	Viscous work after thiazinamium
2,180 (69)‡	2,820	3.5	1.5
2,950 (84)	2,900	3.2	1.5
1,900 (53)	2,380	4.0	2.5
2,600 (68)	2,850	7.0	7.0
2,500 (81)	2,770	ND§	ND
1,700 (64)	2,470	ND	ND
2,600 (68)	3,200	2.5	2.5
2,850 (68)	3,140	2.0	1.8
2,490 (55)	3,000	2.3	0.6
2,720 (80)	3,400	2.4	2.4

over-all bronchodilating effect.

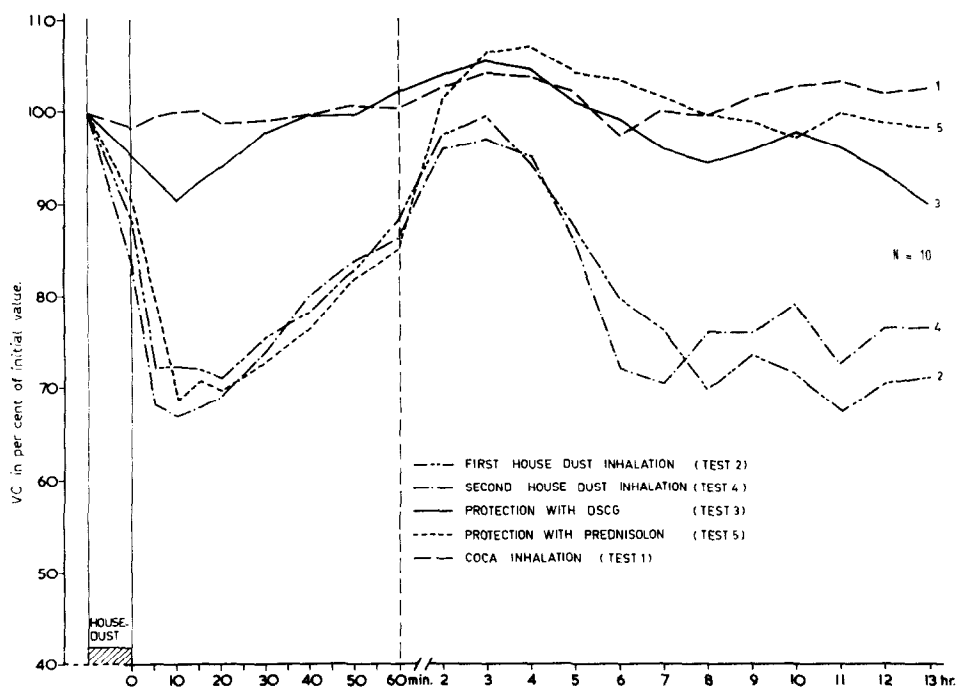


FIG. 2. Mean results of protective effect of DSCG and prednisolone measured with VC (10 patients).

Protective effect of prednisolone

Prednisolone did not give a significant protective effect on the immediate reaction ($p > 0.05$), but it gave an evident protective result on the late obstructive reaction ($p < 0.01$) after house dust inhalation. The maximal fall of the FEV₁ in comparison with Coca's solution inhalation is also to be seen in Fig. 4.

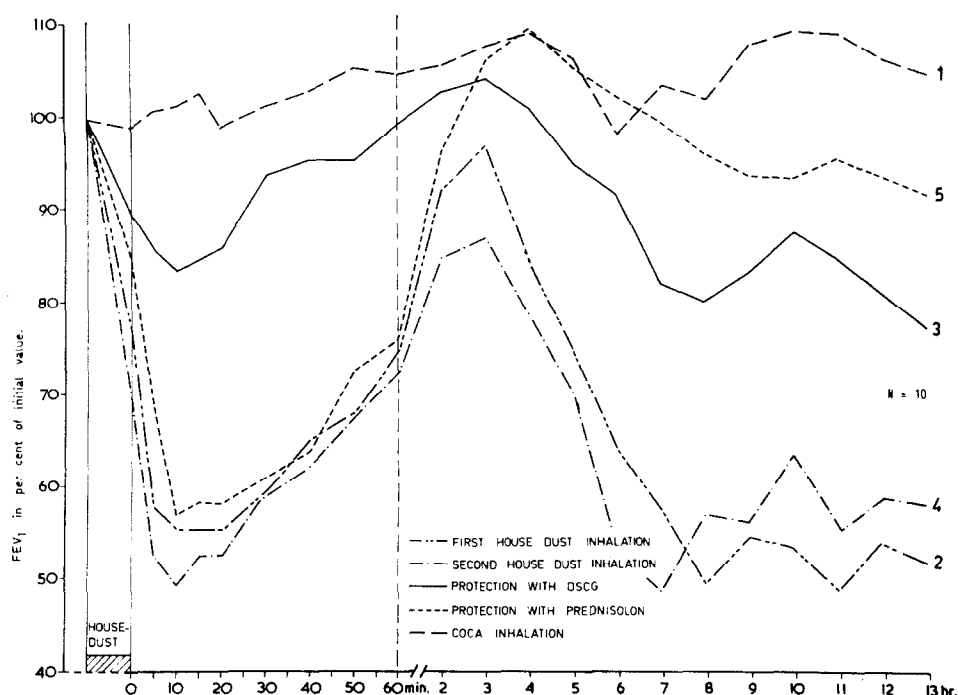


FIG. 3. Mean results of protective effect of DSCG and prednisolone measured with FEV_1 (10 patients).

Protective effect of prednisolone in comparison with the protective effect of disodium cromoglycate

In the late obstructive reaction there was a slightly stronger protective effect with prednisolone than with disodium cromoglycate ($0.02 < p < 0.05$).

Comparison of the two house dust tests

Statistically, there was no difference in the response to house dust challenge between Tests II and IV in regard to both the immediate and the late obstructive reactions ($p > 0.05$), although some patients received metaproterenol for the late obstructive reaction.

DISCUSSION

The present findings demonstrate that both disodium cromoglycate, if inhaled before house dust provocation, and prednisolone have an evident protective effect on the late obstructive reaction. There was, however, a different effect on the immediate reaction: Prednisolone produced no significant effect, whereas disodium cromoglycate gave good protection. We have suggested that by using selective drugs for protection against bronchial allergen challenge we may obtain information about the nature of each reaction.

Immediate reaction

The mechanism of the immediate allergic reaction (type I) in human lung has not been clearly defined, but it is reasonable to assume that bronchial obstruc-

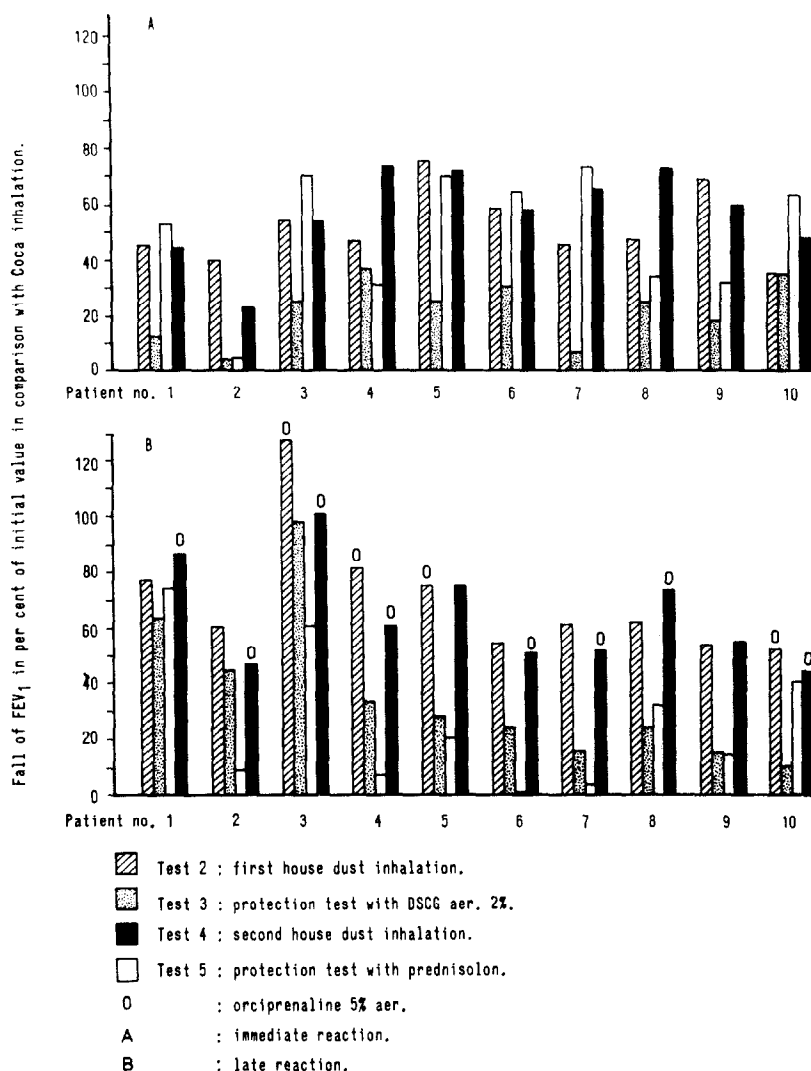


FIG. 4. Maximal fall of FEV_1 in per cent of initial value in comparison with Coca's solution inhalation (also in per cent of initial value) in immediate and late obstructive reactions in each individual patient.

tion is largely attributable to mediators: probably histamine, SRS-A, bradykinin, serotonin, prostaglandins.¹¹

Disodium cromoglycate. DSCG gives in general an evident protective effect, without bronchodilatation, on the immediate reaction,^{2, 12-14} although Herxheimer and Bewersdorff¹⁵ reported that DSCG had only a very moderate protective effect.

Investigations in animals and chopped human lung¹⁶ demonstrated that DSCG has no anti-inflammatory properties, no inhibition of the antigen-antibody union, and no antagonizing action against mediators. Altounyan¹⁷ showed that the fall of FEV_1 after 0.1 per cent histamine aerosol inhalation was decreased after DSCG therapy for 4 weeks. Kerr and associates¹⁸ demonstrated that DSCG

inhaled before intravenously administered histamine diminished the bronchial obstruction. But these patients received DSCG capsules for 2 weeks before the protective test with DSCG, and this treatment, by improving bronchial obstruction, may explain a decrease in histamine reactivity. For this reason the influence of DSCG on intravenously administered histamine remains unresolved. In our experiments no significant effect of DSCG on the histamine threshold value was found in 13 patients.¹⁹

Orr and Cox²⁰ reported that DSCG acts on the enzyme phospholipase A (in rats) known to degranulate the mast cells. But it is not known whether phospholipase A is implicated in the antigen-antibody mast cell degranulation induced in type I hypersensitivity. de Koek²¹ suggested that DSCG might have some alpha-blocking properties. Pharmacologic investigations suggest that DSCG prevents the reaginic antibody-antigen-activated release of histamine and possibly SRS-A from the mast cells.^{16, 20, 22}

Prednisolone. Corticosteroids have little or no effect on type I immediate reactions.²³ There is probably no major influence on the antibody production or on antigen-antibody binding in man.

Corticosteroids have some influence on mediators in animals^{24, 25}; for example, an effect on histamine metabolism, possible prevention of the release of kinins from plasma protein substrate, and a decrease of the penetration of SRS-A (possibly a result of stabilization of the cell membrane). The amount of corticosteroid administered in most animal experiments is much higher than in man.

Salvato²⁶ reported that after treatment with corticosteroids there was a reduction in mast cells in bronchial connective tissue in man.

Late obstructive reaction

This has some features in common with type III reactions, starting at about the same time and also lasting many hours as with the late bronchial obstructive reaction after the inhalation of *Aspergillus fumigatus*.²⁷ There are, however, differences between this late obstructive reaction after house dust and the well-known Arthus reaction. The 10 patients had no leukocytosis, no fever, no crepitations, and no pulmonary infiltrations. On intracutaneous skin testing, 6 patients had a late infiltrative and erythematous reaction, one patient had only a late erythema, and 3 patients had no late reactions. Precipitins could not be demonstrated, but this may be a technical problem. McAllen and associates²⁸ could not find precipitins to house dust mite, *D. pteronyssinus*, in patients with a late obstructive reaction.

Release of enzymes from leukocytes may be an important phenomenon in the type III reaction.^{29, 30} The lysosomal enzymes of the polymorphonuclear leukocytes are sources of mediators involved in the development of inflammation and local vascular injury.³¹

Disodium cromoglycate. It is unsolved how DSCG can have a protective effect on the Arthus reaction¹³ and the late obstructive reaction after house dust inhalation. DSCG does not inhibit histamine release from human leukocytes in vitro,³² though this observation may be more relevant to immediate reactions.

Prednisolone. The mechanism of the effect of prednisolone on the late obstructive

tive reaction is also unknown, but corticosteroids also inhibit the type III reaction.^{33, 34} There is a protective effect on the release of lysosomes from the polymorphonuclear neutrophil leukocytes in the initial stage of the type III reaction. Corticosteroids also increase the resistance of fibrocytes to altered tissue permeability; this is possibly of importance in diminishing the bronchial obstruction.

It is concluded that there seems to be a difference between the mechanism of the immediate and of the late reaction as follows from the fact that prednisolone had no influence on the immediate reaction in contrast to the evident effect on the late obstructive reaction (although it is possible that there is a combination of the late obstructive reaction with the immediate reaction). It is not clear if this late reaction is a pure type III hypersensitivity.

Besides the theoretical problems, the demonstration of a late obstructive reaction and protective activity of these drugs is of practical importance in patients with reversible obstructive lung disease with an allergy to house dust.

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