

Clinical effects of hyposensitization using a purified allergen preparation from Timothy pollen as compared to crude aqueous extracts from Timothy pollen and a four-grass pollen mixture respectively

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Summary

Most extracts used in hyposensitization are complex and ill-defined mixtures of a large number of antigenic components. A highly refined (purified) and well-characterized allergen preparation from Timothy pollen (*Phleum pratense*) is now available.

This paper describes the results of hyposensitization for 3 years comparing the purified preparation Timothy N, the crude extract Timothy O and a four-grass mix in sixty patients with allergic rhinitis due to grass pollen. The sixty patients were randomized into three groups and compared with a control group not hyposensitized. All three groups showed a significant decrease in clinical symptoms compared with the control group. The Timothy N group had a significantly higher nasal tolerance shown by nasal challenge test after 3 years' treatment than the group treated with the crude extract ($P=0.05$). In addition, the Timothy-N-treated patients needed significantly less antihistaminic medication than the patients having received the crude extract or the four-grass mix ($P=0.02$ and $P=0.01$, respectively).

Introduction

Hyposensitization has been shown to be an effective treatment, particularly in pollen allergies, even with the use of crude extracts (Aas, 1977; Lockey & Bukantz, 1976; Norman, 1978; Sørensen, 1976).

In other studies of the effect of hyposensitization, the results reported differ from one investigator to another. Reasons for this may be differences in patients, and in material used for diagnosis and treatment (Aas, 1975; Frostad *et al.*, 1980).

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The quality of the material is critical, but most extracts used are complex and ill-defined mixtures of a large number of antigenic components. Only a few of the antigenic components are of significance for allergy and hence important in immunotherapy (Aas, 1975). Furthermore, the allergenic activity of the extracts are usually not appropriately defined. Many extracts are also more or less unstable with respect to allergenic activity, particularly in the lower concentrations frequently used during hyposensitization.

Another problem is to what extent pollen from one or a few different grasses may suffice in immunotherapy for grass pollen allergies. Only well-characterized and quantitated material and standardized procedures are appropriate for this investigation.

A highly refined (purified) and well-characterized allergen preparation from Timothy pollen (*Phleum pratense*) is now available in a freeze-dried form. This preparation is quantitated with respect to total allergenic activity. It has been shown to represent the crude extract of the same pollen material with respect to allergenic composition and is superior to it when used in the diagnosis of immediate allergy to Timothy pollen (Frostad *et al.*, 1977; Grimmer *et al.*, 1977a; Grimmer *et al.*, 1977b).

The present study compares the purified preparation of Timothy pollen and the corresponding crude extract with regard to efficacy and adverse reactions in prolonged immunotherapy of hay fever, and evaluates if the treatment can be improved by including other relevant grass pollen allergens, in addition to Timothy. In addition, clinical and immunological effects of this immunotherapy were studied in relation to pollen counts.

Materials and methods

Patients

Sixty adult patients from the Oslo region with allergic rhinitis of at least 2 years duration were selected. Patients with perennial rhinitis, atopic dermatitis or asthma, and pregnant women were excluded. All patients included had a well-documented and typical history of intolerable allergic rhinoconjunctivitis with severe symptoms during the grass pollen season (June and July), and were not adequately relieved by standard dosages of disodium cromoglycate, antihistamines and/or topical corticosteroids. They had not previously received immunotherapy. All were shown to have strongly positive skin prick test (SPT) reactions both to the purified and to the crude extract of Timothy pollen at appropriate concentrations (Frostad *et al.*, 1977; Voorhorst & Vankreiken, 1977). Their serum contained immunoglobulin E (IgE) antibodies to Timothy pollen. The patients also had positive skin test reactions and positive (Class 3–4) radioallergosorbent tests (RAST) to all of the other four grass pollen extracts used in testing: cocksfoot (*Dactylis glomerata*), meadow fescue (*Festuca elatior*), ryegrass (*Lolium perenne*) and meadow grass (*Poa pratensis*).

In addition to grass pollen allergy, seven individuals had animal dander allergy (dog) and one allergy to mugwort (*Artemisia vulgaris*). None of the patients were sensitive to pollen from deciduous trees.

Thirty adult patients were selected as a control group (see *design and drop outs*).

Prior to all biological tests all anti-allergic drugs were withdrawn for at least 7 days. Corticosteroids or disodium cromoglycate were not given to the patients receiving injections during the period of observation.

Allergen preparations and extracts

Three different allergen preparations produced by the same manufacturer (Nyegaard & Co. A/S, Norway) were used throughout the studies.

The Timothy O preparation was a crude aqueous extract in Coca's solution.

The Timothy N preparation was a purified allergen preparation containing all the relevant allergens present in a crude extract. Most high molecular weight non-allergenic components and all low molecular weight material were removed by gelfiltration and diafiltration. The preparation was produced from the same batch of pollen as the Timothy O extract and was supplied as a freeze-dried and easily soluble material (Grimmer *et al.*, 1977b).

The grass mix preparation was a crude aqueous extract from equal amounts of four different grass pollens: Timothy (*Phleum pratense*), meadow fescue (*Festuca elatior*), ryegrass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*) in Coca's solution.

The pollens were supplied by Allergen (Engelholm, Sweden).

The solvent and diluent was 0.9% NaCl with 0.5% phenol for skin tests and only saline for nasal tests.

Potency of allergen extracts

The three allergen preparations were biologically standardized. The histamine equivalent in prick testing (HEP) system was used as the reference for allergenic activity. An allergen preparation was said to contain 1000 biological units (BU) per ml when it elicited prick test reactions equivalent to those of a histamine HCl solution 1 mg/ml (5.43 mmol/l) in patients allergic to the allergen extract (Aas *et al.*, 1978).

For the Timothy N preparation, 1000 BU/ml corresponded to 10 µg/ml of the freeze-dried material. The Timothy N preparation was used in ten-fold dilutions from this concentration and for nasal tests also in a 5000 BU/ml concentration. These solutions were used within one week (Frostad *et al.*, 1977).

The Timothy O preparation was used in ten-fold dilutions from a 1:140 w/v concentration. The activity corresponding to 1000 BU/ml of the crude extract was found in a 1:1400 w/v concentration.

The grass mix preparation was used in ten-fold dilutions from a 1:67 w/v concentration. The activity corresponding to 1000 BU/ml of the grass mix preparation was found in a 1:670 w/v concentration.

The crude extracts were stored at +4°C in the highest concentration and dilutions were made the same day as the tests in question were performed.

The refined (purified) preparation retained the allergenic activity unchanged in the freeze-dried state stored at -20°C and in the aqueous solution until used.

To minimize possible effects of glass adsorption all vials were treated in the same way the test being performed within 3 hr of the solvent being introduced.

The allergenic activity of the crude extracts was reduced during the storage and entire study period, to a varying degree and no further data can be given than will be presented under the nasal provocation test.

Design and drop outs

The sixty patients were assigned at random into three groups receiving hyposensitization either with Timothy N (twenty-four patients), Timothy O (seventeen patients) or grass mix (nineteen patients). Immunotherapy was started during March 1975.

Thirty adult patients served as control individuals from late 1976. These patients were selected by the same criteria as those used to select the previous sixty patients. These control individuals had symptoms corresponding in type and severity to those in patients treated with injections as recorded before their treatment was commenced.

The control individuals were not given injections, but were treated with nasal insufflation (approximately every third hour) and conjunctival instillation (four times daily) of disodium cromoglycate during the grass pollination seasons of 1977 and 1978.

The four patient groups were comparable with respect to pre-study history, nasal provocation tests (NPT), SPT, total serum IgE and IgE antibodies before treatment was commenced, as well as to age and sex distribution. Average age in all the patient groups was about 25 years.

The trial was single-blind for the treated groups, i.e. the physician knew which preparation each patient was given, while the patient did not know which preparation he/she was given.

Ten patients from the immunotherapy treated groups dropped out during the trial (Table 1). The numbers and distribution were about similar in each group.

Table 1. The reasons for and number of drop outs in the treated groups

Reason for drop out	Timothy N group	Timothy O group	Grass mix group
Loss of motivation	2	1	1
Outside the Oslo region during the pollen season	2	1	1
Pregnancy	0	1	1

Nasal provocation tests (NPT)

The NPT are described in detail elsewhere (Frostad, 1980; A. B. Frostad, unpubl.). An end-point titration technique with ten-fold dilutions in saline of the Timothy N preparation was employed in all patients. The increasing concentrations were given with an interval of at least 1 week. Identical control tests were performed with saline.

Prior to the tests, excessive nasal secretion was removed by suction during anterior rhinoscopy. The patency of the nasal passages before and after challenge tests was recorded by measuring the bilateral nasal peak expiratory flow rate (PEFR_n). The latter measurement was made by having the patient blow out through both nostrils with the lips tightly closed, into an airtight face mask (A. B. Frostad, unpubl.). The best reproducible result of four or more readings was recorded.

Using direct inspection, two drops of the extract were applied by means of a syringe in both nostrils on the anterior part of the inferior turbinate, 1–2 cm posterior to the front of it (A. B. Frostad, unpubl.).

Reactions were recorded after 20 min by direct inspection and by repeated PEFR_n. The response was considered positive when the patient had a PEFR_n reduction of 25% or more. It was also considered positive if the patient presented with sneezing and

increased secretion and/or swelling of the nasal mucosa, even if their PEFRn was reduced less than 25%.

The threshold dose (TD) of allergen was defined as the lowest dose and/or concentration which caused a positive response. The threshold dose and the PEFRn reduction were used as parameters for assessment of the immunological reactivity of the nasal tissues to allergen challenge. Since stability of allergenic activity could be confirmed only for the refined (freeze-dried) preparation, only this preparation was considered satisfactory for nasal provocation tests.

Skin tests

Skin tests were performed mainly using the SPT and the results were evaluated as described before (Frostad, 1980; Frostad *et al.*, 1977).

In vitro methods

Blood samples were collected immediately before hyposensitization was started and when the first maximum tolerated dose was reached. Samples were also collected before (May) and after (October) the grass pollen season each year, on the same day as the biological tests were performed, but prior to them. All blood samples were kept at room temperature for 2 hr before centrifugation. The serum samples were stored at -20°C .

RAST was performed using Phadebas RAST® Timothy (g6) discs according to the manufacturers instructions (Pharmacia Diagnostics AB, Sweden). The results were calculated as ratios between total count rate of the isotope reagent and the count rate of each disc.

Pollen counts

Throughout the period of treatment the diurnal atmospheric pollen concentration was counted in a Hirst automatic volumetric spore trap during the grass pollen seasons (June 15 to July 30) of 1975, 1976, 1977 and 1978. The trap was placed in an open place

Table 2. Score system for patients' own evaluation of type and severity of symptoms during the grass pollen season

	Sneezing	Blocking of the nose	Nasal secretion	Eye symptoms
0—No symptoms	—	—	—	—
1—Slight symptoms	0–5 attacks of sneezing daily	Mild blocking	Occasional secretion	Mild itching
2—Moderate symptoms	6–15 attacks of sneezing daily	Partly blocked most of the day	Handkerchief has to be used nearly every hour	More troublesome itching and secretion
3—Severe symptoms	More than 15 attacks of sneezing daily	Completely blocked most of the day	Constant secretion	Considerable oedema, itching and secretion

Table 3. Score system for the patients' general impression as to effect of treatment as stated after the grass pollen season in question

Score	Patients' impression
0—Excellent:	Symptom free
1—Very good:	Marked improvement, symptoms only on days with particularly high pollen counts
2—Moderate:	Some relief and obvious change for the better
3—Unchanged	
4—Worse	

near the centre of Oslo at a height of 180 cm above ground level. Other investigations have shown that the pollen counts obtained at this location are representative for pollen counts obtained at other locations within the region inhabited by the patients.

Recording of symptoms

The patient's own evaluation of type and severity of symptoms were recorded each day during the grass pollen seasons. They rated each symptom on a 0–3 scale as shown in Table 2. Their general impression as to effect of treatment was rated on a 0–4 scale (Table 3).

Medication

Medication used to relieve symptoms was restricted to one antihistaminic drug containing a decongestant (brompheniramine) and phenylpropanolamine (Lunarin®). The patients were instructed to take medication only when needed. The number of tablets consumed each day was recorded by the patient. In addition, very restricted (less than 10 ml per season) use of local antihistamine decongestant, antazoline (Antistin-Privin®), was allowed if symptoms were not controlled by the tablets. The patients did not record the use of nasal drops.

Hyposensitization regimen

Hyposensitization was initiated in March 1975 as a rush hyposensitization regimen with the patients hospitalized. All patients followed the same initial injection schedule, for safety purposes starting at 0.1 ml of a 0.1 BU/ml concentration of the Timothy N preparation, 0.1 ml of a 1:10 million w/v (0.14 BU/ml) concentration of the Timothy O preparation and 0.1 ml of a 1:10 million w/v (0.07 BU/ml) of the grass mix preparation. Subsequent doses were increased approximately two-fold (0.1–0.2–0.4 and 0.8 ml) as long as the former dose was well tolerated. The doses were kept constant or even reduced if severe reactions were encountered. A maximum tolerated dose was defined as the one giving a local reaction with erythema and an infiltration of approximately 2–3 cm diameter without local or general symptoms or signs of allergy.

In the rush hyposensitization regimen injections were given four times a day for the first 5 days of treatment, with gradually increasing doses and concentrations, until the maximum tolerated (first maintenance) dose was reached. From this point on,

injections were given at weekly intervals, and then gradually less frequently up to intervals of 4 weeks. If no reactions were observed at this stage, the dosage was again gradually increased at weekly intervals.

From 15 June each year dosage was decreased to 25% of the highest tolerated dose and given every 4 weeks. After 1 October, when airborne grass pollen was not registered any more, the doses were again increased to the highest concentration and dose tolerated.

Adverse reactions

The patients were observed for half-an-hour after each injection. If the injection produced a wheal with diameter less than 4–5 cm or local erythema, this was not considered an adverse reaction.

At each visit the patients were asked if they had observed any reaction to the previous injection.

Statistical methods

Two-sided test procedures have been used to decide whether observed differences are statistically significant. For changes during treatment a Wilcoxon signed rank test was used, and for differences between treatment groups a Wilcoxon rank sum test was used (Lehman, 1975). When the groups were compared with respect to symptom scores, the analysis was based on the average daily symptom scores during the pollen season for each patient.

Results

Reproducibility of NPT

Nasal provocation test was performed with the Timothy N preparation in the control group in 1976, 1977 and 1978. Each patient was tested with the same concentrations each year.

The difference between the PEFR_n reduction in 1977–78 and the PEFR_n reduction in 1976 was calculated for each patient in the Control group. For 1976 and 1977 the largest difference was 6.5%. In 76% of the repeated tests the differences were found to be less than 2%. For 1976 and 1978 the largest difference was 3.9%. In 80% of the tests the differences were less than 2%. This shows that the reproducibility of the NPT technique is very high when performed with the Timothy N preparation.

Stability of the preparations

The results of NPT performed with the Timothy N preparation in the control group may also be used to evaluate the stability of the Timothy N preparation. The difference between the PEFR_n reduction in 1978 and the PEFR_n reduction in 1976 was evaluated for each patient. The mean difference was 0.4% and largest difference was 3.9%. This indicates that the allergenic activity of the freeze-dried Timothy N preparation was stable during these 2 years, confirming previous data from RAST inhibition analyses (Frostad *et al.*, 1977; Grimmer *et al.*, 1977a).

The three preparations were biologically standardized before the study and the concentrations were adjusted to give identical allergenic activities in skin testing. In 1978 NPT was performed in the Control group using the three preparations. The

Table 4. NPT in the Control group ($n=25$) performed with the three preparations in 1978. The listed values are mean reductions in PEFRn

Threshold dose (BU/ml)	<i>n</i>	Timothy N preparation (%)	Timothy O preparation (%)	Grass mix preparation (%)
10	2	28	25	20
100	9	36	32	26
1000	14	38	32	23

results are given in Table 4. The observed differences between the preparations are statistically significant (All $P < 0.001$).

Patients with an observed PEFRn reduction at one TD had no reduction at the preceeding TD. Assuming a linear relationship between log dose and PEFRn reduction, the observed differences indicate that the Timothy O and grass mix preparations in 1978 exhibited 70 and 50% respectively of the original allergenic activities.

Number of injections and total dosage of allergen

The number of injections were almost the same for all patients. The total dosage of allergen given during the trial varied considerably between the patients, and was lower in the Timothy N group than in the Timothy O group and the grass mix group (Table 5).

Adverse reactions

No adverse reactions were observed during the treatment period in any of the patients.

Pollen counts

The pollen counts varied considerably on a day-to-day basis during the pollen seasons and between seasons (Figs 1, 2 and 3). The average pollen counts during the pollen seasons in 1975, 1976, 1977 and 1978 were 21, 17, 6 and 12 ct/m³ respectively.

Table 5. Total allergen dose (BU) given in the three patient groups during the hyposensitization regimen 1975–1978. The doses were evaluated before the pollen season each year

		Timothy N group	Timothy O group	Grass mix group
1st year	Median	13000	12000	16000
	Range	4000–21000	4000–75000	4000–63000
1st and 2nd year	Median	38000	98000	95000
	Range	7000–105000	7000–312000	13000–208000
1st, 2nd, 3rd year	Median	103000	276000	238000
	Range	14000–317000	11000–447000	53000–329000

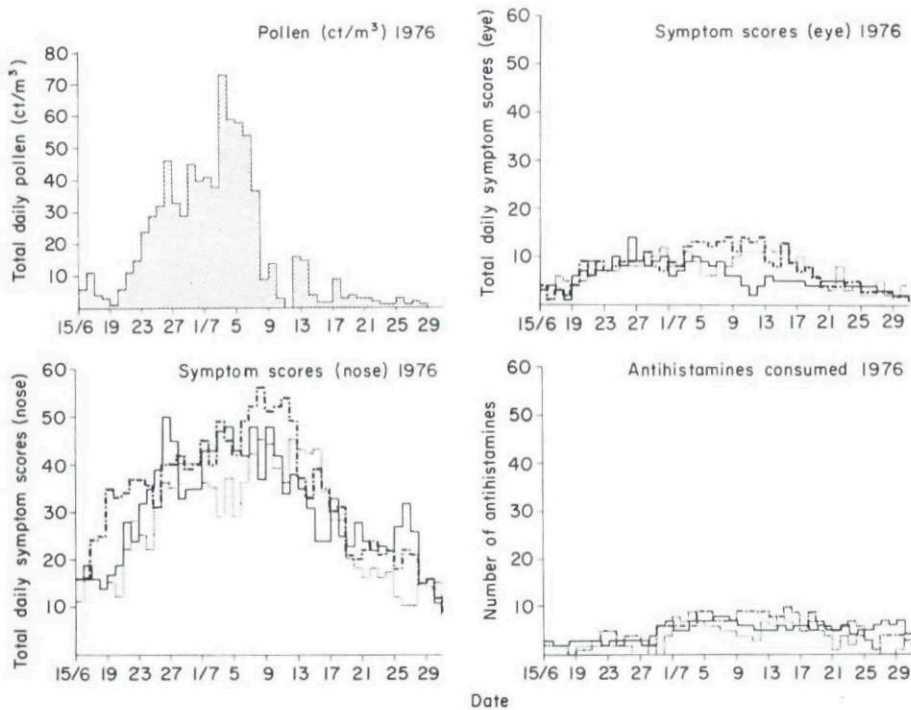


Fig. 1. Total daily symptom scores and use of antihistamines as correlated to daily atmospheric grass pollen counts (ct/m³) air, for the three groups receiving immunotherapy, after 1 year of treatment (1976). -.-.-, Grass mix group; —, Timothy O group; ·····, Timothy N group.

Relation between pollen counts and symptom scores

Figures 1, 2 and 3 record the relationship between daily pollen counts and patients symptom scores in the treated groups and the control group. The figures show that there was a good concordance between pollen counts and symptom scores reported in each group.

The differences between the treated groups and the control group as regards symptom scores and consumption of antihistamines became apparent during the second and third pollen season.

Figures 2 and 3 show that the differences between the groups were largest when the pollen counts were high, and that the differences became less apparent as the pollen counts dropped at the end of the seasons.

Nasal symptoms

Whilst the median of nose and eye symptom scores in the treated groups and the control group during the pollen seasons are shown in Fig. 4 for the statistical evaluations the nasal symptoms were treated alone.

In 1976 the observed differences between the groups were not statistically significant.

In 1977 the observed differences between the Timothy N group and the control group were statistically significant ($P=0.02$), but the differences between the other group pairs were not.

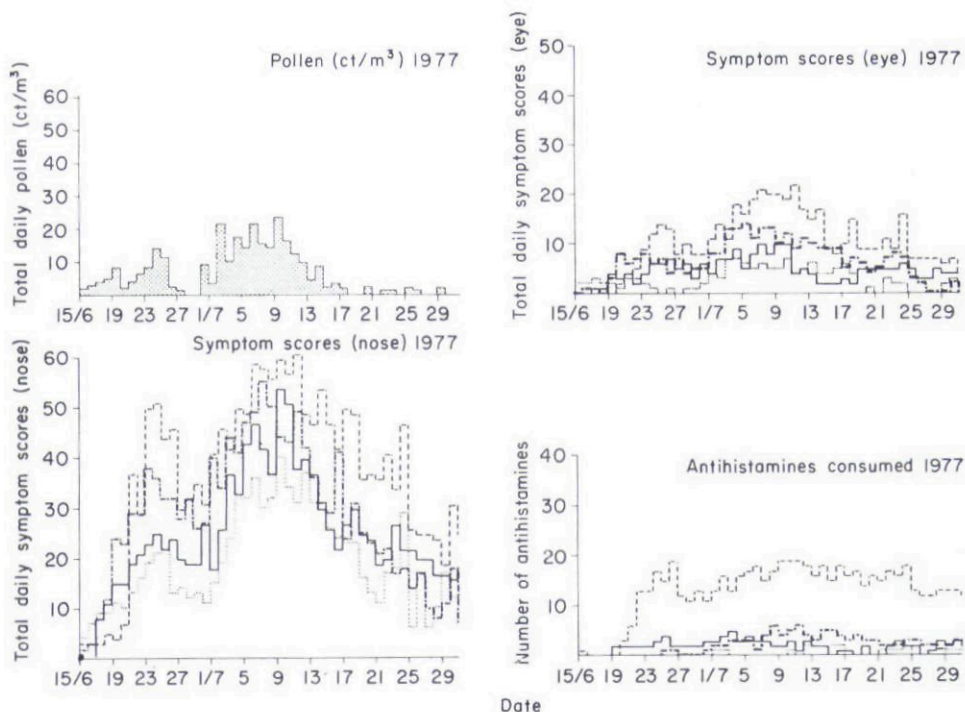


Fig. 2. Total daily symptom scores and use of antihistamines as correlated to daily atmospheric grass pollen counts (ct/m^3) air, for the three groups receiving immunotherapy and control group during the grass pollen season 1977. -----, Control group; -.-.-., grass mix group; —, Timothy O group; ·····, Timothy N group.

After 3 years of treatment the observed differences between the control group and the treated groups were statistically significant (Timothy N: $P < 0.001$; Timothy O: $P = 0.006$; grass mix: $P = 0.01$). The other observed differences in 1978 were not statistically significant.

Eye symptoms

For the statistical evaluations the eye symptoms were treated separately.

In 1976 the observed differences between the groups were not statistically significant.

In 1977 the observed differences between the control group and the Timothy N and Timothy O group were statistically significant (Timothy N: $P < 0.001$; Timothy O: $P = 0.04$), but the other observed differences were not statistically significant.

After 3 years of treatment the observed differences between the control group and the Timothy N and Timothy O group were statistically significant (Timothy N: $P < 0.001$; Timothy O: $P = 0.02$). The observed difference between the Timothy N group and the grass mix group was also statistically significant ($P = 0.01$). The other observed differences in 1978 were not statistically significant.

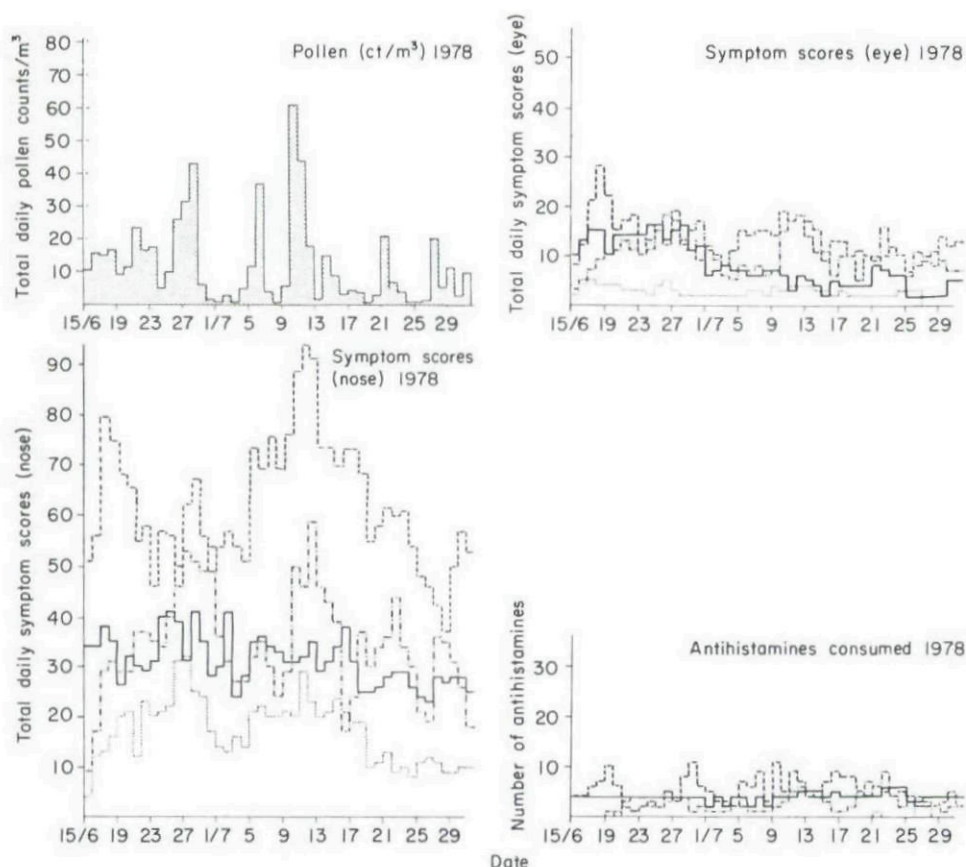


Fig. 3. Total daily symptom scores and use of antihistamines as correlated to daily atmospheric pollen counts (ct/m^3) air for the three groups receiving immunotherapy and control group during the grass pollen season 1978. — — — —, Control group; — · — ·, grass mix group; — — —, Timothy O group; · · · ·, Timothy N group.

Consumption of antihistamines

The number of patients using antihistamines in the treated groups and the Control Group during the pollen seasons are shown in Fig. 4.

In 1976 the observed differences between the groups were not statistically significant.

In 1977 the observed differences between the control group and the treated groups were statistically significant (Timothy N: $P < 0.001$; Timothy O: $P = 0.006$; grass mix: $P < 0.001$). The other observed differences in 1977 were not statistically significant.

After three years of treatment the observed differences between the Timothy N group and the other groups were statistically significant. (Control: $P < 0.001$; Timothy O: $P = 0.02$; grass mix: $P = 0.01$).

Patient self-evaluation

The average scores of patient self evaluation in the treated groups were reduced in 1977 as compared to 1976. The Timothy N and Timothy O groups had further reduced

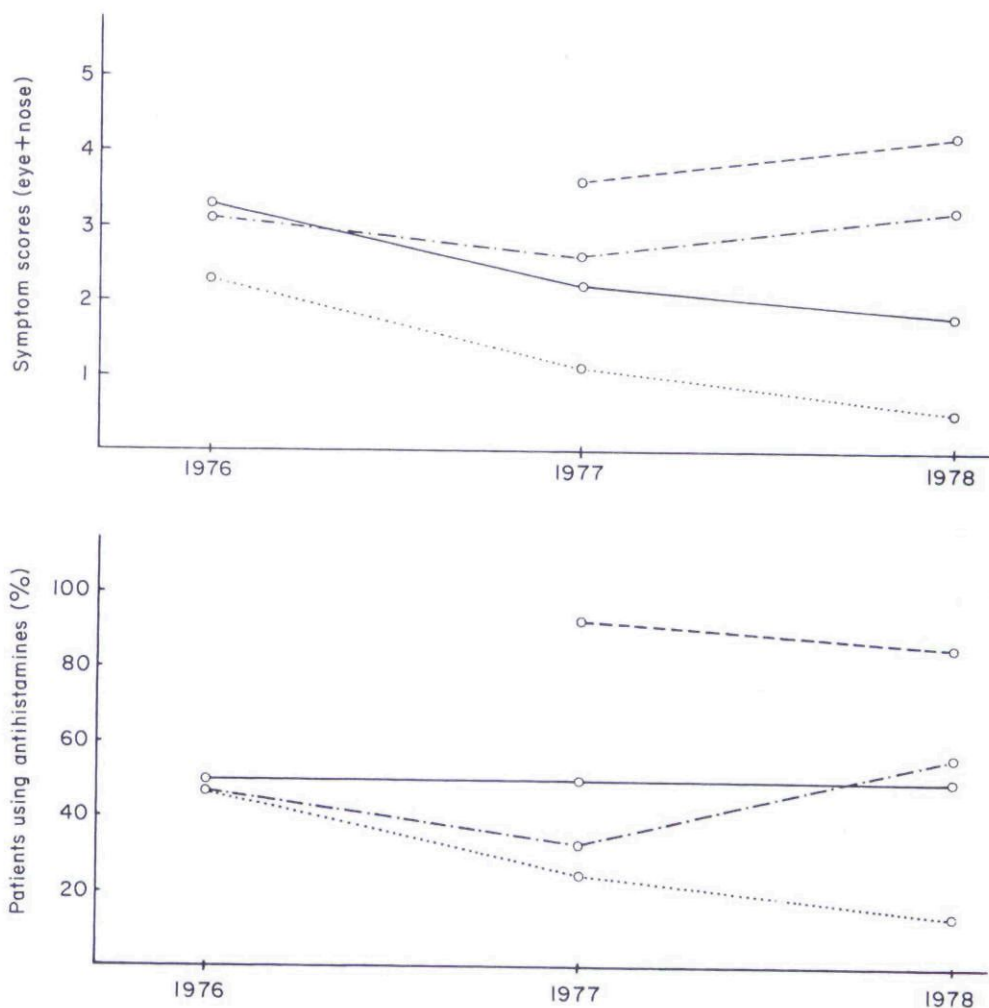


Fig. 4. Median symptom scores (nose and eye) and consumption of antihistamines during the grass pollination season in 1976, 1977 and 1978. -----, Control group; -.-.-, grass mix group; —, Timothy O group; ·····, Timothy N group.

values in 1978, while the average scores of patient self evaluation were increased in the grass mix and control groups.

In 1976 the observed differences between the groups were not statistically significant.

In 1977 the following observed differences were statistically significant (Timothy N/control: $P < 0.001$; grass mix/control: $P = 0.02$; Timothy N/Timothy O: $P = 0.01$).

After 3 years of treatment all the observed differences except the observed differences between the Timothy O and the grass mix group, were statistically significant (Timothy N/Timothy O: $P = 0.002$; Timothy N/grass mix: $P = 0.005$; Timothy N/control: $P < 0.001$; Timothy O/control: $P < 0.001$; grass mix/control: $P = 0.02$).

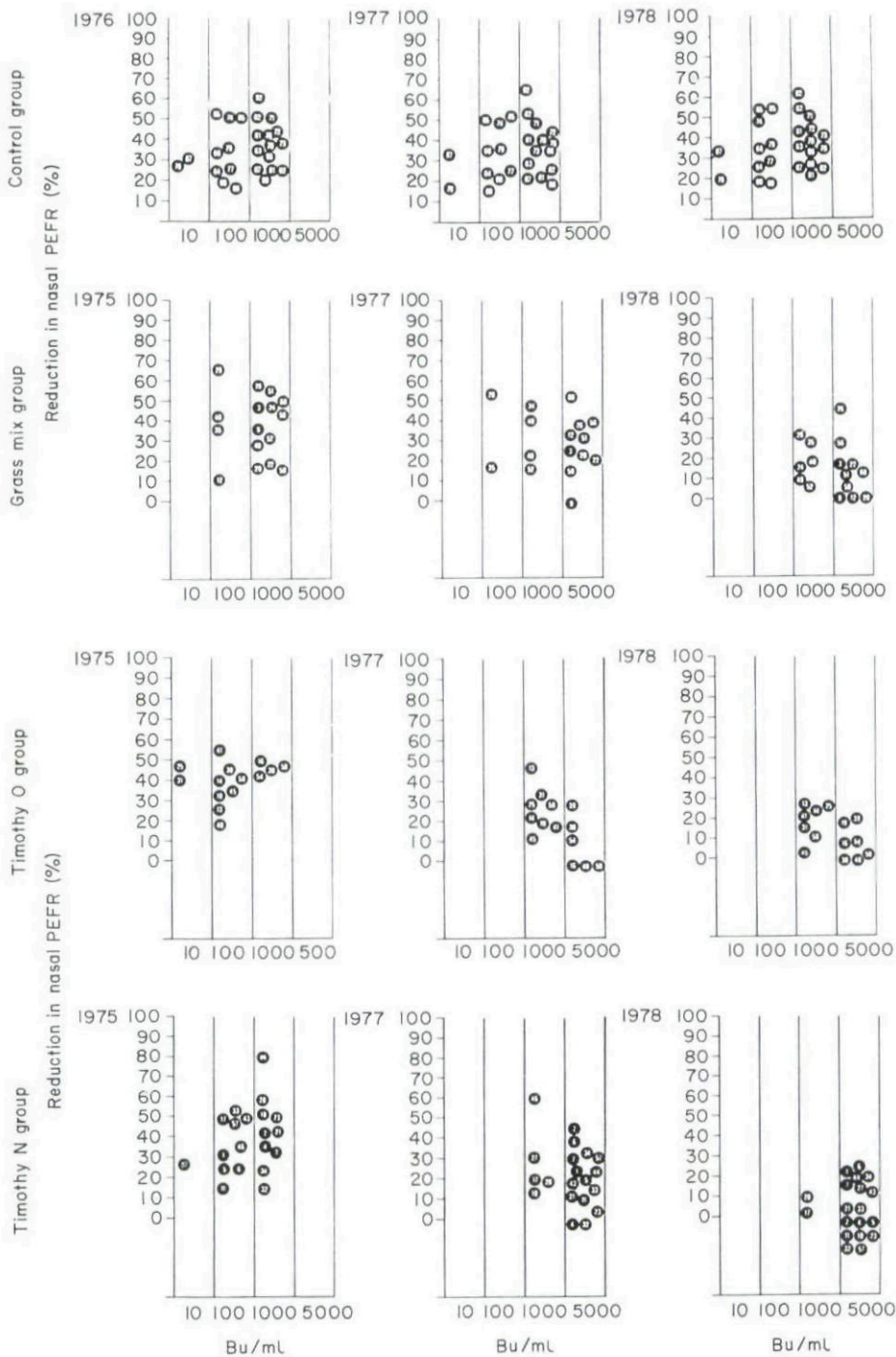


Fig. 5. Nasal provocation tests (NPT) using the Timothy N preparation. Allergen threshold dose (TD) and resulting reduction in PEFRn (%) are indicated before and after 2 and 3 years of treatment (for each individual indicated by a given number) in the three groups given immunotherapy and after 2 years of observation in control group.

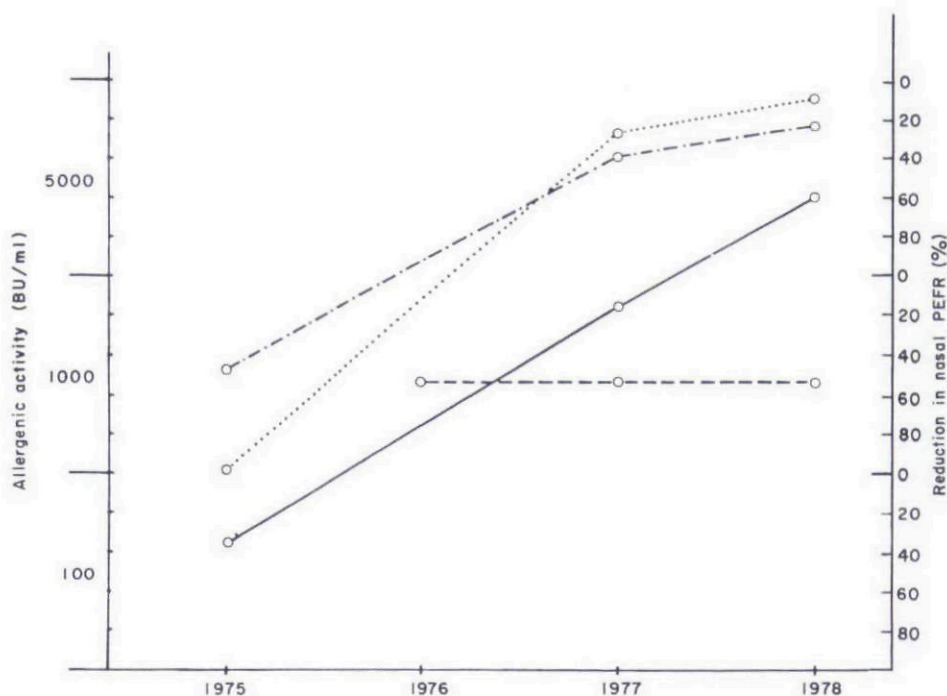


Fig. 6. Median scores of nasal provocation tests (NPT) provoked by Timothy N preparation before and after 2 and 3 years of treatment in the three groups given immunotherapy and after 2 years of observation in Control group. —, Control group; — · —, grass mix group; —, Timothy O group; · · ·, Timothy N group.

Nasal tolerance

Figures 5 and 7 show the threshold doses and the PEFRn reductions in the treated groups and control group in 1975, 1976, 1977 and 1978, respectively. The median scores of NPT performed with the Timothy N preparation are shown in Fig. 6.

In 1977 the observed differences between the control group and the treated groups were statistically significant ($P < 0.001$ for each of the groups).

After 3 years of treatment the observed differences between the control group and the treated groups were statistically significant ($P < 0.001$ for each of the groups). The observed differences between the Timothy N group and the Timothy O group were also statistically significant ($P = 0.05$).

The median and range of the scores of NPT performed with the Timothy O and the grass mix preparation in 1978 are given in Tables 6 and 7. The observed differences between the groups and the corresponding P values in Table 6 and 7 are almost identical with those found when NPT was performed with the Timothy N preparation.

Correlation between symptom scores and total dose of allergen

The relations between total dose of allergen and average daily symptom scores during the pollen seasons in 1978 for the treated groups were calculated (results not shown).

In the Timothy N group there seemed to be a correlation between these two parameters, i.e. high allergen doses seemed to correspond to low average symptom scores.

In the Timothy O and the grass mix group such a correlation did not seem to exist.

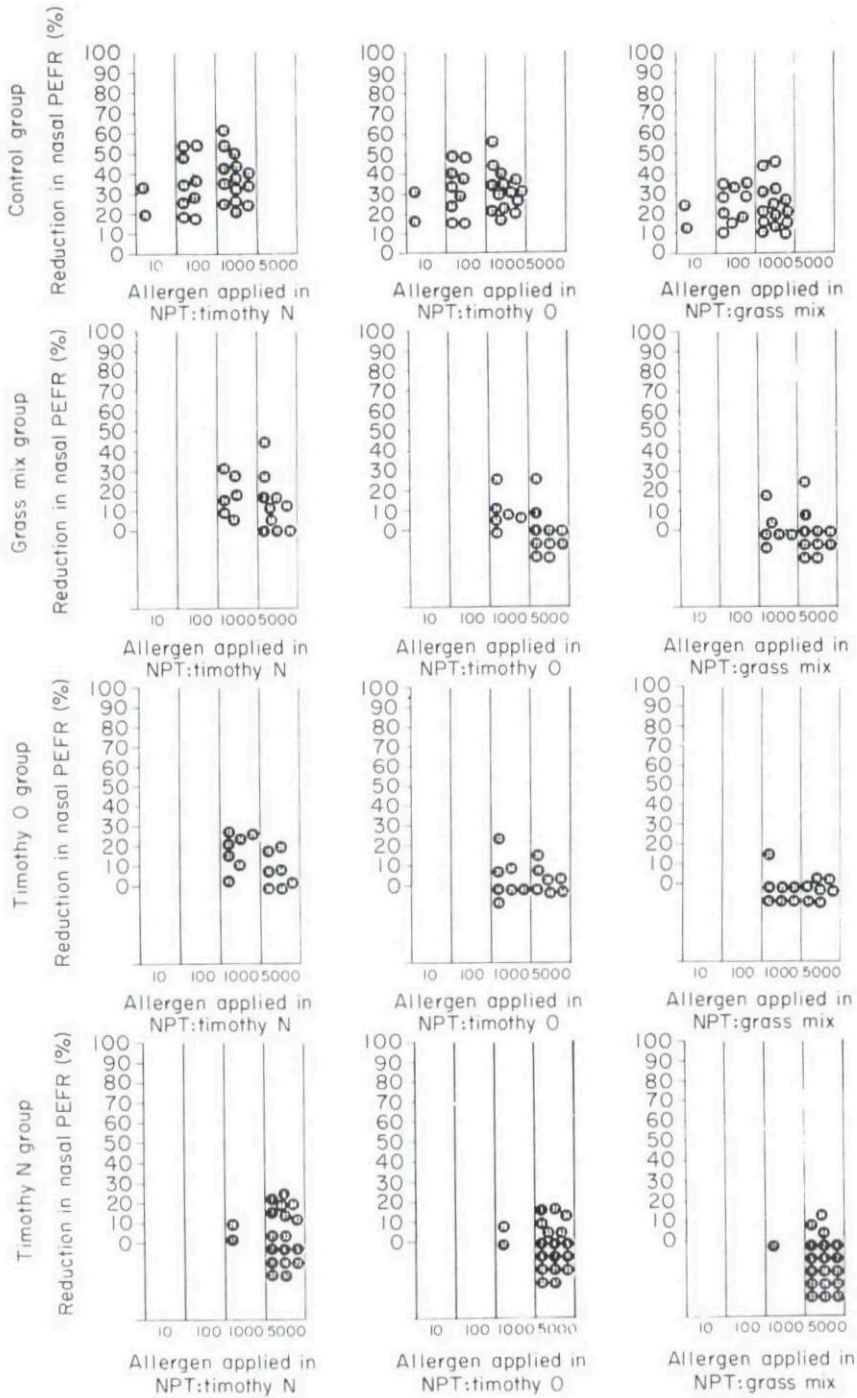


Fig. 7. Allergen threshold dose (TD) and resulting reduction in PEFRn (%) for each individual (indicated by a given number), when performing NPT using Timothy N preparation, Timothy O preparation and grass mix preparation. The results were obtained after 3 years of immunotherapy and after 2 years of observation in control group.

Table 6. NPT performed with the Timothy O preparation in 1978. The listed values are mean reductions in PEFR_n

	Timothy N group	Timothy O group	Grass mix group	Control group
Median	5 HEP, 0%	5 HEP, 58%	5 HEP, 5%	1 HEP, 45%
Range	1 HEP, 60%– 5 HEP, 0%	1 HEP, 25%– 5 HEP, 0%	1 HEP, 25%– 5 HEP, 0%	0.01 HEP, 33%– 1 HEP, 19%
<i>n</i>	20	14	16	25

Table 7. NPT performed with the Grass mix preparation in 1978. The listed values are mean reductions in PEFR_n

	Timothy N group	Timothy O group	Grass mix group	Control group
Median	5 HEP, 0%	5 HEP, 56%	5 HEP, 5%	1 HEP, 44%
Range	1 HEP, 60%– 5 HEP, 0%	1 HEP, 15%– 5 HEP, 0%	1 HEP, 19%– 5 HEP, 0%	0.01 HEP, 25%– 1 HEP, 10%
<i>n</i>	20	14	16	25

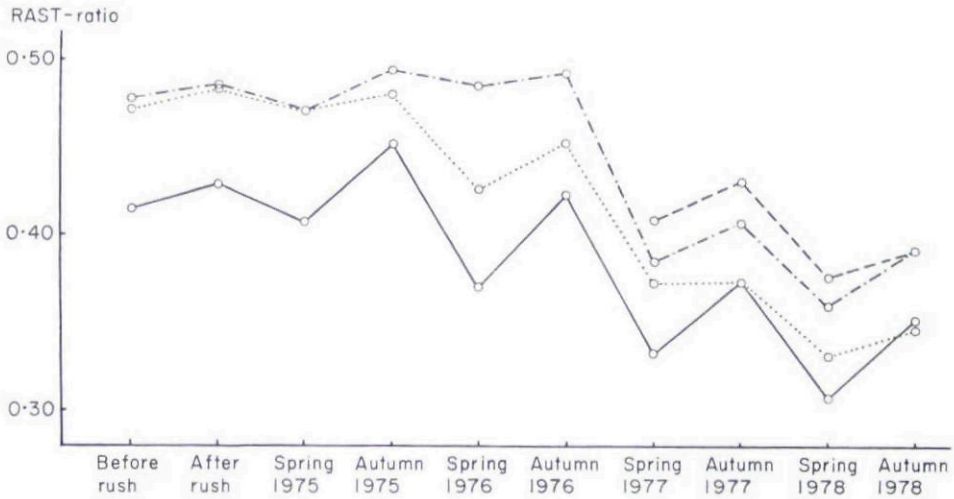


Fig. 8. Average RAST-ratios before and after rush hyposensitization and before and after the pollen seasons in 1975, 1976, 1977 and 1978. — — — —, Control group; — · — ·, grass mix group; —, Timothy O group; · · · ·, Timothy N group.

Allergen-specific IgE antibody levels

Allergen-specific IgE antibody levels were tested by Phadebas RAST using Phadebas Timothy discs. The tests were performed in consecutive runs at the end of the study for better comparison of results. The average RAST ratio in the treated groups and the control group before and after rush hyposensitization in 1975, and before and after the pollen seasons in 1975, 1976, 1977 and 1978, are given in Fig. 8. There was no significant rise in average RAST ratio during the first months of treatment in any of the groups. Characteristic preseasonal to post-seasonal increases in average RAST ratio were maintained throughout the treatment.

The observed reduction in average RAST ratio from before rush hyposensitization in 1975 to before the pollen season in 1978 are statistically significant for all the treated groups ($P < 0.001$ for each of the groups). The observed differences between the groups before the pollen season in 1978 are not statistically significant.

The average RAST ratio of the control group in 1977 and 1978 are higher than the mean values of all the treated groups.

Discussion

In studies of the effect of hyposensitization, the results reported differ from one investigator to another. One reason for this may be that the quality of allergen extracts may vary considerably from study to study, and even from time to time and batch to batch during each study (Aas, 1975). On this basis our purpose was to compare the effects of a purified and a crude preparation from the same original source and with defined allergenic activities. It was not our aim to study the effect of immunotherapy as such, hence placebo controls was not used in this trial. Furthermore, it was not considered ethically acceptable to give placebo injections for 3 years, since there is substantial evidence for good clinical effects from hyposensitization with pollen extracts (Lockey & Bukantz, 1976; Norman, 1978; Sørensen, 1976).

The purified, well-characterized and standardized preparation from Timothy pollen used here has previously been shown to represent the corresponding fresh, crude pollen extract with respect to contents of allergenic components and total activity (Frostad *et al.*, 1977; Grimmer *et al.*, 1977a; Grimmer *et al.*, 1977b). A refined preparation of this quality should be preferable to a crude extract for hyposensitization therapy. Theoretically, the demonstration that the purified preparation is superior for diagnostic purposes, does not necessarily imply that it is also superior in therapy. This study shows, however, that the purified preparation is more effective than the crude extract in immunotherapy. This confirms that the refined preparation contains sufficient concentrations of the clinically important allergens found in Timothy pollen. The somewhat better results obtained with the refined preparation may be due to a more favourable antigen competition situation and stability of the preparation.

In this investigation emphasis was put on results of NPT as the only appropriate objective measure available.

We found the direct investigation of the nasal function following titrated allergen challenge suitable for evaluation of the efficacy of specific hyposensitization. The use of an identical allergen preparation before and after treatment is mandatory for this kind of evaluation. This study has shown that the allergenic activity of the refined allergen preparation was the same throughout the study period, and the good reproducibility of the results achieved in the NPT performed in the control individuals indicates a good reproducibility of the NPT itself. After 3 years of treatment all patients, both the

treated groups and the control individuals showed a higher nasal tolerance when the crude preparations were used in NPT, than when the purified preparation was used in NPT. This is in agreement with observations that aqueous preparations lose some allergenic activity during storage. Results obtained in prolonged therapy studies with such extracts are not reliable.

There is no standardized and generally accepted way to express the degree of nasal tissue reactivity to allergen challenge (Mygind, 1978; Okuda, 1977). Parameters to be considered are concentration and dose of allergen introduced and degree of reaction to that particular dose. The site of application (Okuda, 1977) and general status and non-specific lability of the tissues may be critical (Mygind, 1978). The time interval between introduction and assessment is important. In the present study attempts were made to obtain standard conditions for techniques and allergen dose, site and mode of application, time interval, measurement and recording of symptoms, and resistance to nasal air flow. On this basis, the use of TD and PEFRn reduction as indication of nasal tissue reactivity to the allergens seemed rational, although there may be reservations concerning details.

The stability of the allergenic activity of an allergen extract is of decisive importance when the extract is used to evaluate the efficacy of hyposensitization (Aas, 1977). This has not been sufficiently considered in the literature on this subject. The availability in the present study of a stable, freeze-dried and purified allergen preparation which is appropriately standardized, fulfills the clinical demands for a modern therapeutic allergen preparation and facilitates investigations of the kind reported. The NPT's performed in the control individuals in this study confirm the allergenic stability of the preparation used.

The symptoms scores reported were in agreement with the NPT observations. No significant differences between the groups were found after only 1 year of treatment as shown in Fig. 1, also confirming that the groups were adequately matched. Correlations between pollen exposure and symptoms are essential for the evaluation of the symptom scores recorded.

There are many drawbacks to counting atmospheric pollen at a single site. Nevertheless, it gives an impression of the degree of exposure in the region surrounding that site (Norman, 1974; Norman, 1978). It has been shown that the pollen counts found at the sampler site in Oslo are largely representative for those found at three other sites 50–150 km distant from Oslo.

The pollination around Oslo is heaviest during the months of June and July and less heavy in May and September. During June and July the pollen counts are dominated by pollen from grasses. This period of time thus offers a fine opportunity to study the clinical response to a specific pollen without significant interference and overlap from other pollen sources. In the three pollen seasons studied symptom scores correlated well with pollen counts from year to year. This was particularly marked in the control individuals and in patients treated with a grass mix preparation. During the second summer under study the pollen counts were much lower than during the previous season. The symptom scores and amount of medication used during the second summer would probably be lower than the year before, irrespective of treatment. Without reference to pollen counts and without objective assessment of the allergenic tolerance threshold this would easily have led to biased interpretations of the results of any treatment. During the third grass pollen season the atmospheric pollen counts per m³ air was higher than in the previous year. Both average daily symptom scores during

and self evaluation after the third pollen season showed a marked increase when the amount of pollen again increased. The use of symptom score and drug consumption diaries may have a limited reliability due to their subjective character, but the results were confirmed by the objective assessment.

The study indicates also that the Timothy pollen allergens cross-react with the most important ones of other grass pollens with respect to allergens responsible for the symptoms in question. This is in correspondence with other *in vitro* and *in vivo* studies (Löwenstein, 1978; Morrow Brown, Thantrey & Jackson, 1979).

The influence on allergen specific IgE (RAST) and total IgE concentration (PRIST) of hyposensitization therapy has been reported in several publications and has been reviewed by Norman (Norman, 1978). Recent studies employing grass pollen extracts (Deuschl, Johansson & Fagerborg, 1977; Foucard & Johansson, 1976; Foucard & Johansson, 1978; Kjellman & Lanner, 1980) report an increase of allergen-specific IgE antibodies during the first months of therapy and then a decrease as treatment was continued.

The average values of Timothy-specific IgE antibodies showed no or only slight increases during the first months of therapy. Reasons for this difference from the cited studies are unknown. After 3 years the average values were significantly reduced in all the treated groups. In agreement with a recent study (Kjellman & Lanner, 1980), but contrary to others (Norman, 1978) it was observed that the characteristic pre-seasonal to post-seasonal increases in average values were maintained throughout the treatment. Although there were significant differences between mean symptom scores of the treated groups in 1978, the average values of the *in vitro* parameters did not exhibit any significant differences. Neither the objective nor the subjective improvements caused by hyposensitization are reflected by these parameters in the present study.

The mechanisms by which these immunologic changes occur in hyposensitization treatment have not been elucidated. There also seems to be a lack of clinical correlations in studies of the interactions between T and B lymphocytes and macrophages in the production of antibody (Rocklin *et al.*, 1980; Gershon, 1980).

Numerous investigations of hyposensitization therapy have suggested that the clinical effect seems related to the total dose of antigen given (Norman, 1978). In this study the total dose was calculated as the amount of biological units of active allergen and not as the total amount of protein of which the main part is irrelevant. For this calculation only the refined freeze-dried and allergenically stable preparation is adequate.

The total dose given during the period was to a large extent determined by the maximum tolerated dose for each individual. Relatively high doses were given, both with the crude and with the purified extract. The total dose given in the three patient groups during the first year of hyposensitization regimen was almost identical for the purified preparation and the crude extracts. After the second and third year the total doses of crude Timothy allergen extract and the grass mix preparation, although showing marked variability, were up to three times higher for most patients when compared to the refined preparation. A part of this effect may be due to reduction in allergenic activity of the crude preparations during storage. This reduction has not been taken into consideration when calculating the given total doses (BU) since no exact values could be obtained for all the vials used.

Other studies have shown that hyposensitization with crude pollen extracts has

specific beneficial effects in 75–80% of patients as compared to improvement in approximately 30% of patients given placebo (Sørensen, 1976). Our study of grass-pollen-allergic patients confirmed this, and showed that those treated with the purified allergen preparation or with aqueous crude extract of timothy pollen and an extract of four-grass mixture were found to have less allergic symptoms than a comparable control group treated with disodium cromoglycate without injections. The patients receiving hyposensitization were also shown to acquire higher specific tolerance of the nasal tissues whereas non-hyposensitized controls had principally unchanged nasal sensitivity to repeated challenges with the Timothy pollen preparation with unchanged allergenic activity.

A hyposensitization study comparing another partly purified preparation with two separated major allergens from Timothy pollen has recently been published (Østerballe, 1980). The results in question are not considered conclusive (Frostad, 1981) but indicate that a partly purified preparation may be more effective than separate allergens. This suggests that purification should not be carried too far for therapeutic extracts.

This study demonstrates that the purified, representative and stable allergen preparation used is at least as effective in immunotherapy as the crude extracts from the same original source. A purified preparation is preferable to others because characterized and stable allergen preparations increase the safety of hyposensitization treatment.

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Appendix

Purified allergen preparations analogous to the Timothy N described here was further developed and later introduced as Spectralgen® in some countries. The 1000 BU as reported in this paper is equivalent to 5000 BU in the commercially available preparation.

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