

Hyposensitization

Comparing a Purified (Refined) Allergen Preparation and a Crude Aqueous Extract from Timothy Pollen

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Most extracts used in hyposensitization (immunotherapy) are complex and ill-defined mixtures of a large number of non-antigenic and antigenic components, only a few of the latter being of significance for allergy and allergen specific immunotherapy. A new purified and well-characterized allergen preparation from timothy pollen is now available, and it has been shown to be superior to the corresponding crude aqueous extract in the diagnosis of IgE-mediated human allergy to timothy pollen.

This paper describes the results of hyposensitization for 2 years, with the purified preparation and the crude extract compared. The changes in *in vivo* and *in vitro* tests following this treatment in 40 patients with allergic rhinitis due to grass pollen are reported.

Both patient groups showed a significant decrease in clinical symptom scores when compared with a control group during the grass pollen season. For all groups the symptom scores correlated well with atmospheric pollen counts. Nasal challenge tests showed a significant increase in nasal tolerance to timothy pollen after 2 years of treatment, but nasal tolerance was unchanged in the control group. Nasal function as a criterion for evaluating the effect of specific hyposensitization is discussed.

Serum concentrations of timothy pollen-specific IgE antibodies showed a significant decrease for the group treated with the purified preparation and a slighter and non-significant reduction for the other treated group. There was no significant change in total serum IgE levels.

The results indicate that the purified preparation is preferable to the crude aqueous extract in hyposensitization.

Key words: allergen extracts; allergens; hyposensitization; immunotherapy; pollen allergy.

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Hyposensitization (immunotherapy) was first introduced as treatment for pollen hay fever in 1911 (4, 14). Since then the treatment has been carried out in a large number of patients, and will probably be continued. The clinical value of the treatment is still a subject of debate (2, 15, 16). According to the scientific literature, good therapeutic results are obtained, provided that the diagnosis is correct and that sufficiently high

doses of an appropriate allergen preparation are given for a prolonged time (2, 16, 19).

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 (1). Furthermore, the allergenic activity of the extract is 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. Allergen extracts may also contain toxins, endotoxins and irritants interfering with both diagnosis and therapy (1). This makes many extracts quite unreliable.

A highly purified and well-characterized allergen preparation from timothy pollen (*Phleum pratense*) is now available. The allergenic activity of this preparation is quantitated in an appropriate manner. It has been shown to represent the crude extract and is superior to it when used in the diagnosis of immediate allergy to timothy pollen (6, 8, 9). The purpose of the present study was to compare the purified preparation and the crude extract of timothy pollen as regards its efficacy in therapy.

PATIENTS AND MATERIAL

Patients

Forty-one 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 disodiumcromoglycate, 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 test concentrations (6). Their serum contained immunoglobulin E (IgE) antibodies to timothy pollen. The patients had also positive skin test reactions and positive (class 3–4) radioallergosorbent tests (RAST) to all of the other four grass pollens

also used in testing (*Dactylis glomerata*, *Festuca elatior*, *Lolium perenne* and *Poa pratensis*).

The patients were assigned at random into two groups, receiving hyposensitization either with the purified preparation (Timothy N) or the crude extract (Timothy O). Thirty adult patients with allergic rhinitis due to timothy pollen served as control individuals from early 1977. None of the control subjects had previously received hyposensitization. The clinical data of the control individuals corresponded in type and severity to those of the patients treated with injections, as recorded before the treatment was commenced. They were not given any injections, but were treated with nasal insufflation and conjunctival installation of disodiumcromoglycate during the whole grass pollination season of 1977. Otherwise they were on the same restricted drug regimen as the patients receiving hyposensitization.

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

Allergen preparations and extracts

Two different allergen preparations produced from the same original pollen material were used (Nyegaard & Co. A/S, Oslo, Norway). Timothy N is a highly refined allergen preparation supplied as a freeze-dried and easily soluble material (9). Timothy O is a crude aqueous extract in Coca's solution. The two preparations were comparable with respect to allergenic activity as assessed by means of skin prick testing. For this purpose, the Histamine Equivalent in Prick testing (HEP) system was used as the reference for allergenic activity. An allergen preparation was said to contain 1,000 Biological Units (BU) per ml, when it elicited prick test reactions equivalent to those of the prick tests with a Histamine HCl solution 1 mg/ml (5.43 mmol/l) in most patients allergic to the substance (3).

For Timothy N, 1,000 BU/ml corresponded

to 10 µg/ml of the substance. The N preparation was used in 10-fold dilutions from this concentration and for nasal tests also in a 5,000 BU/ml concentration. These solutions were used within 1 week. The crude extract was used in 10-fold dilutions from a 1:140 weight to volume (w/v) concentration. The activity corresponding to 1,000 BU/ml of the crude extract was found in a 1:1,400 w/v concentration (6, 7). All crude extracts were used before the expiration date given by the manufacturer. The stability of the allergen preparations was studied by means of skin and nasal tests in the control individuals and by RAST inhibition tests as described under Experiments and Results in this paper.

Allergosorbents

Discs with the Timothy N preparation were produced as described before (8, 21). Phadebas Timothy (g6) discs were purchased from Pharmacia AB, Uppsala, Sweden.

Patient serum

Serum was 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.

METHODS

Skin tests

Skin tests were performed mainly using the prick test method (SPT). In a number of patients, also intracutaneous tests (ICT) were carried out. The results were evaluated as described before (6). All patients were skin tested at the same time of the day and year, prior to the start of immunotherapy and after 2 years of treatment. The reproducibility of the SPT technique was assessed in 10 of the control individuals by performing duplicate prick tests with the Timothy N pre-

paration (1,000 BU/ml) and the histamine reference with an interval of 2 years.

Nasal provocation tests (NPT)

All nasal provocation tests were performed in the same way as described elsewhere (5), using an end point titration technique with 10-fold dilutions of the Timothy N preparation in all patients. The different dilutions 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 the nose with the lips tightly closed, into an airtight face mask (5). 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 bilaterally on the anterior part of the inferior turbinate, 1–2 cm posterior to the front of it (5).

Reactions were recorded after 20 min by direct inspection and by repeated PEFR_n. The response was considered to be positive when the patient presented with sneezing, increased secretion and/or swelling of the nasal mucosa, and a reduction in nasal PEFR_n of 25% or more. It was also considered positive with less than 25% reduction of PEFR_n if the patient showed very prominent clinical reactions with the above symptoms.

The threshold dose (TD) of allergen was defined as the lowest concentration and dose which caused a definite response. For the final assessment the threshold dose (TD) was used to indicate the immunological reactivity of the nasal tissues to allergen challenge.

The precision and reproducibility of the nasal provocation test with the material and method used were assessed by repeating the

Table 1

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

test after 1 year in the non-treated control subjects. All repeated tests were performed at the same time of year and same time of day as the first one.

Paper radioimmunosorbent test (PRIST)

Phadebas® PRIST was performed according to the manufacturers instructions. (Pharmacia AB, Uppsala, Sweden.)

Radioallergosorbent test (RAST) and RAST inhibition

RAST was performed as described by Pharmacia AB. For the statistical evaluations, ratios between total count rate of the isotope and the count rate of each disc were calculated. RAST-inhibition was performed as previously described (8, 21).

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 1976 and 1977. The trap was placed on an open place in the centre of Oslo at a height of 180 cm above ground level.

Recording and evaluation of symptoms

The patients' own evaluation of the type and severity of symptoms and registration of medication taken were obtained from day-

to-day records as well as through the patients' own impression before and after the grass pollen seasons in question. The patients were asked to rate each symptom on a 0–3 scale as shown in Table 1. Their general impression as to the effect of treatment was rated on a 0–4 scale (Table 2).

Medication used to relieve symptoms was restricted to one antihistaminic drug containing a decongestant (brompheniramine and phenylpropanolamine (Lunerin®)). The patients were instructed to take medication only when needed. The number of tablets used each day was recorded. 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, however. Attempts were made to correlate the scores for symptoms and drugs used with the pollen counts.

Table 2

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

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

Statistical methods

All statistical analyses were based on non-parametric methods. In situations concerning changes during treatment, a two-sided Wilcoxon signed mid-rank test was used. For comparison of Timothy-N and Timothy-O, a two-sided Wilcoxon mid-rank-sum test was used. Median and range were used as indexes of magnitude and dispersion, respectively (11). (Median: The observation (or the mean of the two observations) having an equal number of observations below and above the figure in question.)

EXPERIMENTS AND RESULTS

Stability of allergen preparations

Both SPT, NPT and RAST inhibition were used to control the stability of the allergenic activities of the preparations used throughout the study period.

The duplicate and repeated skin tests in the control individuals demonstrated that the activity of Timothy N was stable. There was no reduction in SPT reactions following hyposensitization. The nasal provocation tests using the same batch of the purified allergen

preparation in the control individuals confirmed these results. No clinically significant difference could be observed between the repeated tests in the same individuals, although there were considerable variations between the individual patients (Table 3).

RAST inhibition experiments

A freeze-dried reference preparation of Timothy N was kept at -20°C . To evaluate the stability of the preparations used for hyposensitization and biological tests, new solutions of the same concentrations were produced from the reference preparation at the end of the experiments. All the preparations were then tested in parallel using RAST-inhibition. Changes in activity of the stored preparations were less than $\pm 20\%$ which is within the reproducibility limits of this method (8).

Reactions to diagnostic test

Diagnostically, the NPT's performed demonstrated marked individual differences in degree of specific nasal tissue reactivity to allergen challenge. The results of skin testing and/or RAST could not be used to predict this in the individual patient.

Table 3

Stability of allergenic activity of Timothy N as demonstrated by nasal provocation tests (NPT). The reduction in nasal peak expiratory flow rate (PEFRn) following the threshold dose (TD) of allergen challenge was measured with an interval of 1 year

No.	Control patient	April 1976		April 1977	
		TD in HEP	Reduction in PEFRn (%)	TD in HEP	Reduction in PEFRn (%)
26	S.J.	5.0	43.4	5.0	50.0
29	M.S.	1.0	24.0	1.0	24.0
31	F.H.	1.0	25.0	1.0	21.9
33	G.B.	1.0	37.5	1.0	40.0
35	K.H.	5.0	40.0	5.0	40.0
36	T.R.S.	1.0	25.0	1.0	25.9
41	M.Aa.	5.0	37.8	5.0	37.5
43	S.R.	5.0	25.8	5.0	21.8
44	B.J.	5.0	50.0	5.0	53.1
48	G.J.	1.0	31.5	1.0	36.3

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 purified allergen preparation, or 0.1 ml of a 1:10 million w/v (0.14 BU/ml) concentration of the crude extract. 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 infiltrate of approximately 2–3 cm in diameter without focal 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.

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

After 1 year of treatment and just before the grass pollen season of 1976, the total dose given ranged from 3,700 BU to 20,800 BU with a median of 12,300 BU for the purified preparation, and from 4,100 BU to 75,000 BU with a median of 17,400 BU for the crude extract. After 2 years of treatment the corresponding total pre-seasonal median doses were 47,300 BU (7,100 BU–105,300 BU) for the purified preparation, and

136,100 BU (7,100 BU–311,700 BU) for the crude extract.

Three patients (2 in N, 1 in O group) were excluded after 1 year of treatment because of loss of motivation, and three patients (2 in N, 1 in O group) were excluded because they moved to other places during the pollen season and one because of pregnancy. Thirty-four out of the 41 patients completed the study (20 in the Timothy N group and 14 in the Timothy O group).

Effects on symptoms

The pollen counts during the grass pollen season varied considerably on a day-to-day basis, and there was a marked difference in the amounts of pollen measured during the two seasons (Fig. 1). During the first pollen season after the start of hyposensitization there was no consistent difference between the two groups as regards symptom scores or use of antihistamines (Fig. 2). There was a

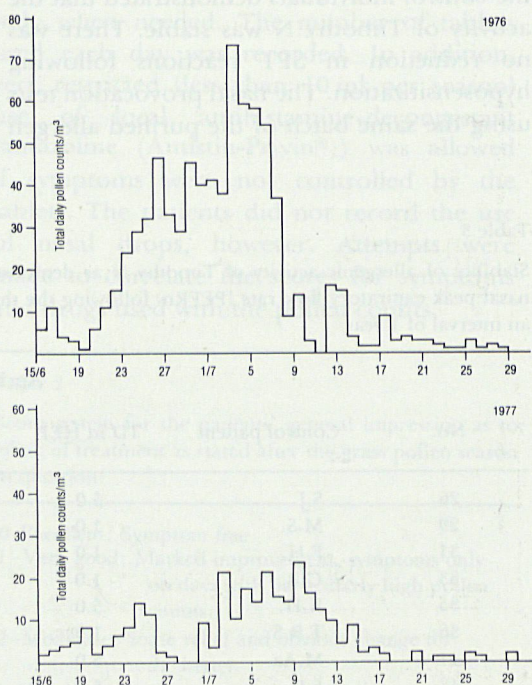


Fig. 1. Atmospheric pollen counts at same site in Oslo during the grass pollen season of 1976 and 1977.

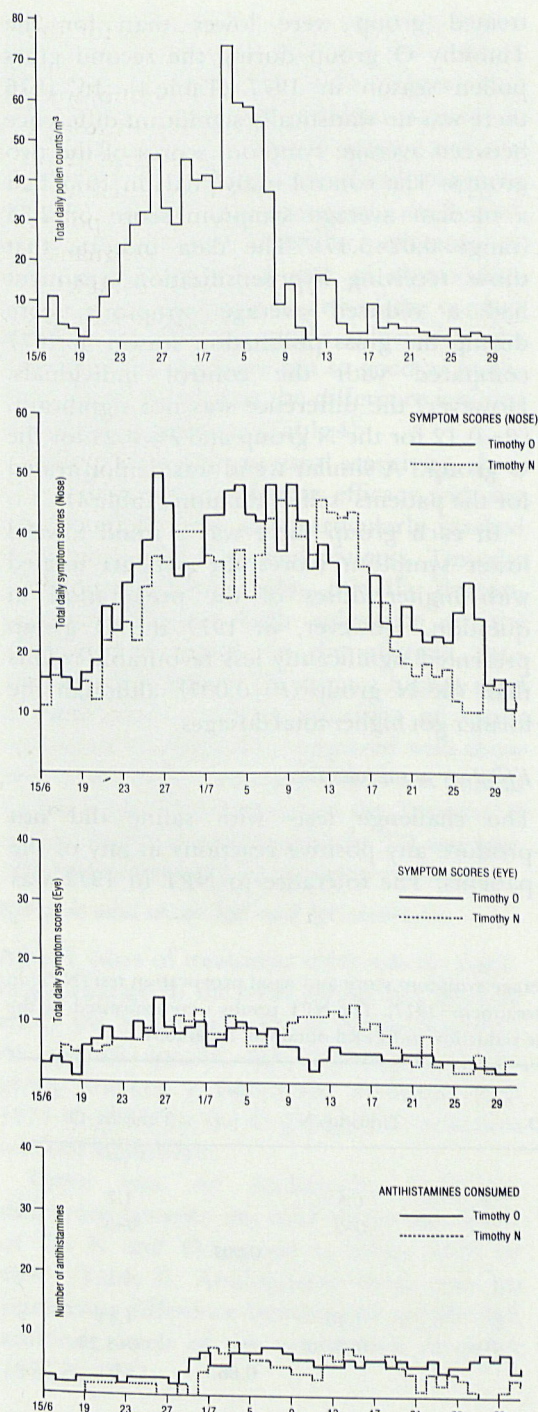


Fig. 2. Total daily symptom scores and use of antihistamines as correlated to daily atmospheric grass pollen counts/m³ air for the two groups receiving immunotherapy, after 1 year of treatment (1976).

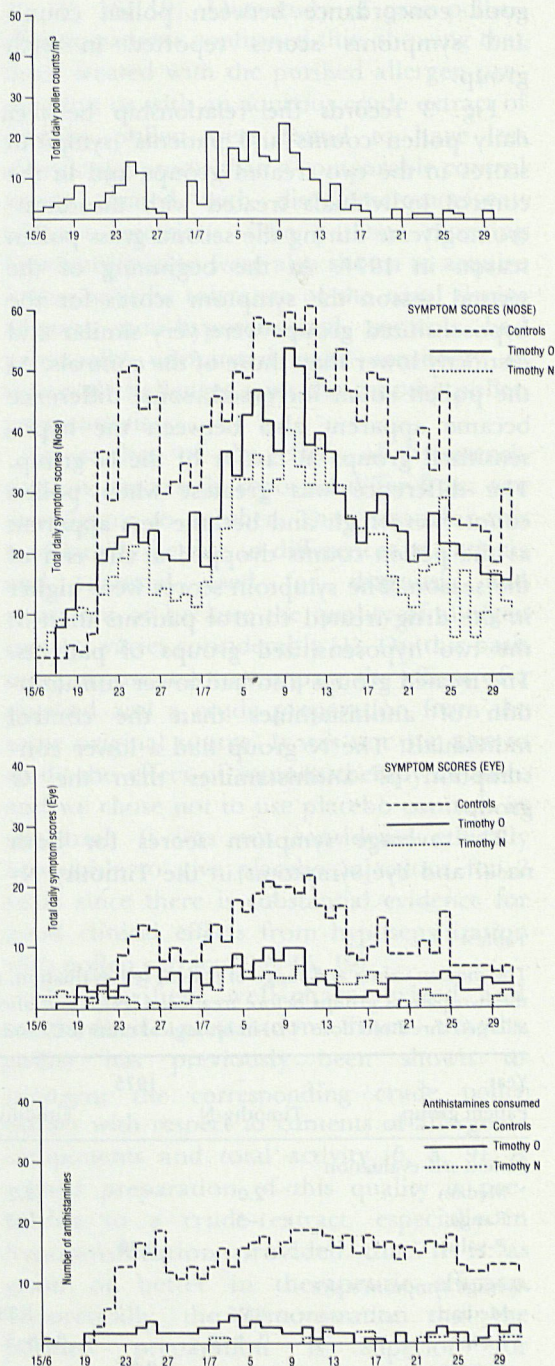


Fig. 3. Total daily symptom scores and use of antihistamines as correlated to daily atmospheric grass pollen counts/m³ air, for the two groups receiving immunotherapy and control group on disodium cromoglycate during the grass pollen season of 1977.

good concordance between pollen counts and symptoms scores reported in each group.

Fig. 3 records the relationship between daily pollen counts and patients' symptom scores in the two treated groups and in the control individuals treated with disodium-cromoglycate during the second grass pollen season in 1977. At the beginning of the second season the symptom scores for the hyposensitized groups were very similar and distinctly lower than those of the controls. As the pollen count increased, some difference became apparent also between the hyposensitized groups in favour of the N group. The difference was greatest when pollen counts were high and became less apparent as the pollen counts dropped at the end of the season. The symptom scores were higher in the drug-treated control patients than in the two hyposensitized groups of patients. The treated groups also had lower consumption of antihistamines than the control individuals. The N group had a lower consumption of antihistamines than the O group.

The average symptom scores for both nasal and eye symptoms in the Timothy N-

treated group were lower than for the Timothy O group during the second grass pollen season in 1977 (Table 4). In 1976 there was no statistically significant difference between average symptom scores of the two groups. The control individuals in 1977 had a median average symptom score of 2.38 (range 0.02–5.17). The data indicate that those receiving hyposensitization treatment had a reduced average symptom score during the grass pollination season in 1977 compared with the control individuals. However, the difference was not significant. ($P=0.12$ for the N group and $P=0.23$ for the O group.) A similar trend was demonstrated for the patients' self evaluation (Table 4).

In each group there was a trend toward lower symptom scores for patients treated with higher doses of the preparation in question. However, in 1977 the O group presented significantly less favourable results than the N group ($P=0.007$), although the former got higher total dosages.

Effect on nasal tolerance

The challenge tests with saline did not produce any positive reactions in any of the patients. The tolerance to NPT in 1977 was

Table 4

The median values and range of patient self-evaluation, average symptom score and nasal provocation test (NPT) in the two patient groups before treatment (1975) and after treatment (1977). The NPT results were expressed by the allergen threshold dose (TD) in biological units (BU) and the reduction in PEFRn obtained, separately

Year	1975		1977	
Patient group	Timothy-N	Timothy-O	Timothy-N	Timothy-O
Patient self-evaluation				
Median	2.0	2.2	0.8	1.7
Range	1–3	1–3	0–2	0–3
<i>P</i> -value	0.39		0.007	
Average symptom score				
Median	2.06	1.83	1.40	1.81
Range	0–4.19	0.68–5.17	0–5.70	0–3.28
<i>P</i> -value	0.84		0.66	
NPT				
Median	1,000 BU/ml, 97%	100 BU/ml, 34%	500 BU/ml, 27%	1,000 BU/ml, 15%
Range	10 BU/ml, 28%	10 BU/ml, 40%	1,000 BU/ml, 60%	1,000 BU/ml, 45%
	– 1,000 BU/ml, 15%	– 1,000 BU/ml, 43%	– 5,000 BU/ml, 0%	– 5,000 BU/ml, 0%
<i>P</i> -value	0.23		0.42	

significantly higher for those receiving hypo-sensitization treatment than for the control individuals ($P < 0.001$ for both comparisons) (Figs. 4 and 5). In nine patients (patients 4, 11, 21, 23 and 32 in the N group, and 16, 48, 54 and 59 in the O group) full tolerance of the highest concentration used had developed, whereas a similar reduction in nasal sensitivity after 1 year of observation could not be observed in any of the control patients. In NPT the results achieved in the Timothy O group were not so good as in the Timothy N group, but the difference was not statistically significant (Table 4).

Also, with respect to nasal sensitivity, there was a trend towards higher efficacy with the highest doses. This was particularly marked in the Timothy N-treated patients. Timothy N-treated patients, who initially demonstrated relatively high tolerance to the allergen preparation in the allergen provocation test, had to be given a higher total antigen dose to obtain an effect of hypo-sensitization treatment, compared with those with low initial tolerance (Fig. 6). A similar trend could not be found in the Timothy O group. In the control group the TD was consistently unchanged on repeated challenges.

Effect on total serum IgE and IgE antibodies

After 2 years of treatment there was no significant change in the total serum IgE level of either the N group or the O group (Table 5). As regards specific IgE antibodies, the N group showed a significant decrease from 1975 to 1977. In the O group this reduction was not significant.

There was no statistically significant difference between the total serum IgE levels of the N and O groups in either 1975 or 1977 (Table 5). Analogously there was no significant difference between the specific IgE antibody levels of the two groups in either 1975 or 1977.

DISCUSSION

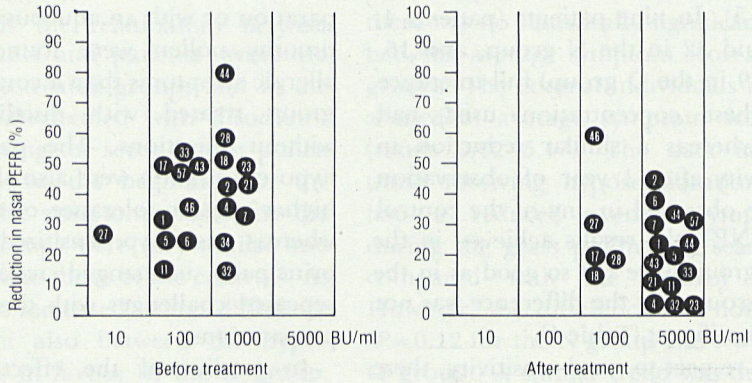
Hyposensitization has been shown to be an effective treatment, particularly in pollen allergies, even with the use of crude extracts

(2, 12, 16, 19). Our study of grass-pollen-allergic patients confirmed this, showing that those treated with the purified allergen preparation or with an aqueous crude extract of timothy pollen were found to have less allergic symptoms than a comparable control group treated with disodiumcromoglycate 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 preparations.

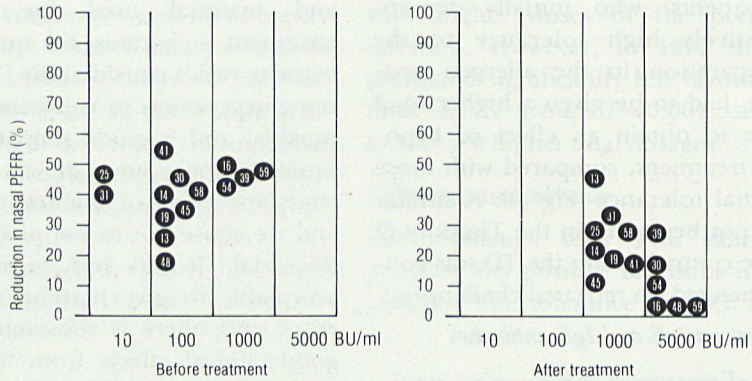
In studies of the effect of hyposensitization, the results reported differ from one investigator to another. The reason for this may largely be due to differences in patients and material used for diagnosis and treatment or because the quality of allergen extracts varies considerably (1). On this basis our purpose was to compare the effects of a purified and a crude preparation from the same original source. It was not our aim to study the effect of immunotherapy as such, and we chose not to use placebo controls in this trial. It was not considered ethically acceptable to give placebo injections for 2 years since there is substantial evidence for good clinical effects from hyposensitization with pollen extracts (12, 16, 19).

The purified, well-characterized and standardized preparation from timothy pollen has previously been shown to represent the corresponding crude pollen extract with respect to contents of allergenic components and total activity (6, 8, 9). A refined preparation of this quality is preferable to a crude extract, especially in hyposensitization provided that it is as good or better in therapeutic efficacy. Theoretically, the demonstration that the purified preparation is superior for diagnostic purposes, does not necessarily imply that it is also superior in therapy. The results of this study indicate, however, that the purified preparation is very effective in immunotherapy as well, and this confirms that it has the same major and clinically

TIMOTHY N



TIMOTHY O



CONTROLS

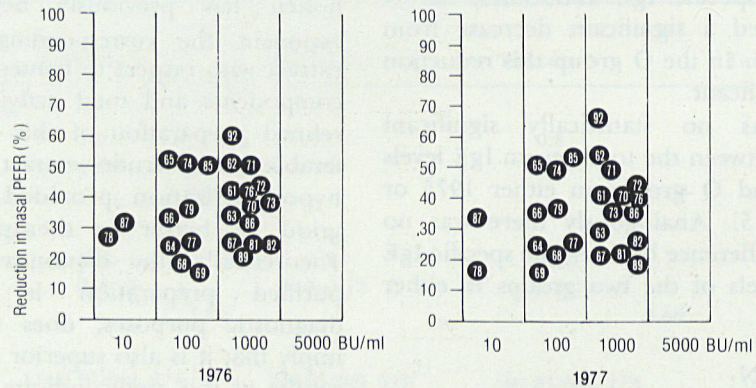


Fig. 4. Allergen threshold dose (TD) and resulting reduction in PEFRn (%) before and after 2 years treatment for each individual (indicated by a given number) in the two groups given immunotherapy.

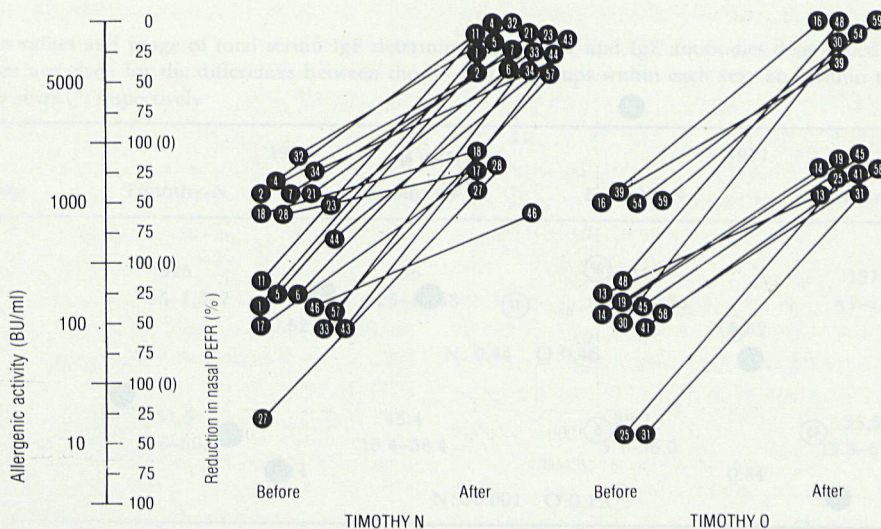


Fig. 5. Allergen threshold dose (TD) and resulting reduction in PEFRn (%) before and after 2 years treatment for each individual (indicated by a given number) in the two groups given immunotherapy and after 1 observation year in control group on disodium-cromoglycate.

important allergenic activities as found in timothy pollen.

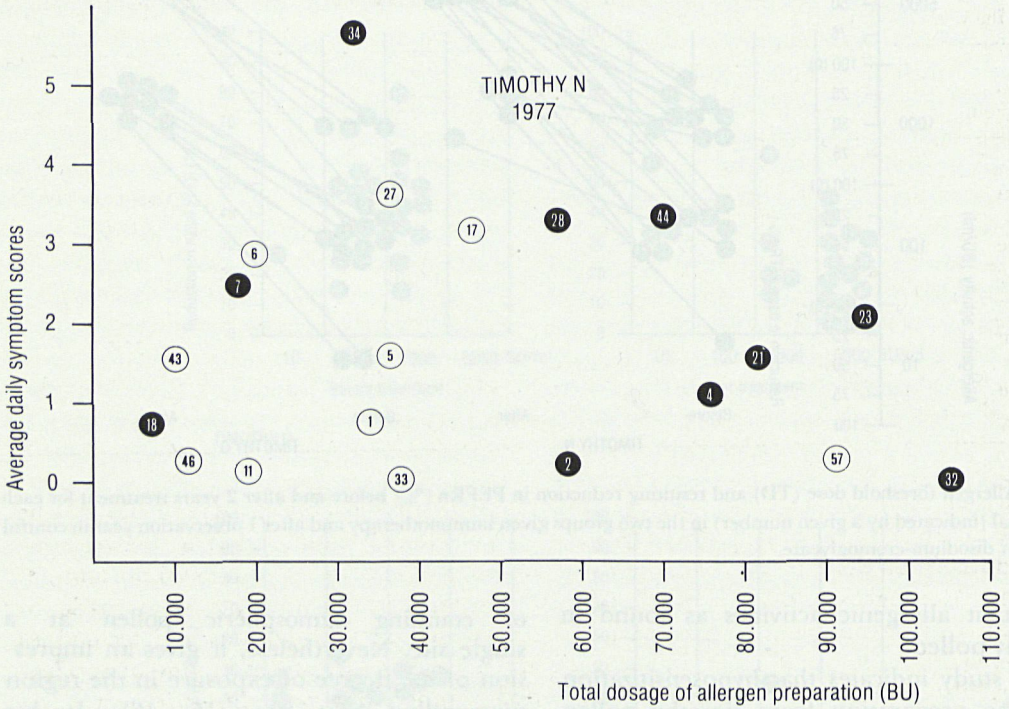
The study indicates that hyposensitization with the preparation from timothy pollen causes symptomatic improvement during the grass pollen season. This indicates that the timothy pollen preparations cross-react with the most important ones of other grass pollens with respect to allergens responsible for the symptoms in question.

The stability of the allergenic activity in the allergen extract used is of decisive importance for the use of skin test or nasal provocation test threshold doses as a criterion for evaluation of the efficacy of the specific hyposensitization (2). 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 fulfils the clinical demands for a modern therapeutic allergen preparation and facilitates investigations of the kind reported. The skin tests and nasal provocation tests performed in the control individuals in this study confirm the allergenic stability of the preparation used.

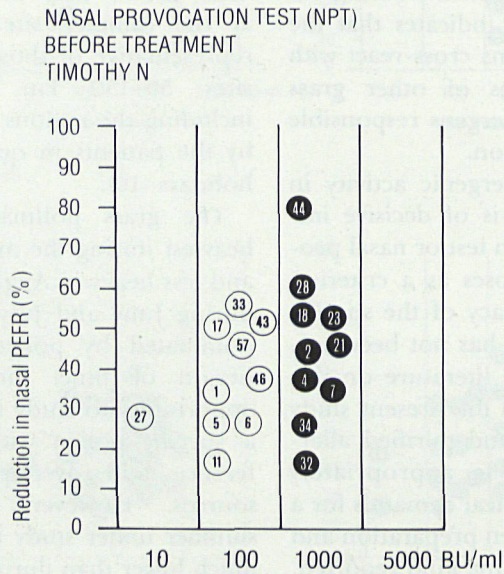
There are many limitations to the value

of counting atmospheric pollen at a single site. Nevertheless, it gives an impression of the degree of exposure in the region surrounding that site (15, 16). It has been shown that the pollen counts found at the sampler site in Oslo are largely representative of those found at three other sites 50–150 km distant from Oslo, including the regions most frequently visited by the patients in question during summer holidays (10).

The grass pollination around Oslo is heaviest during the months of June and July and less heavy in August and September, and during June and July the pollen counts are dominated by pollens from grasses. This period of time, therefore, offers a fine opportunity to study the clinical response to a specific pollen without significant interference and overlap from other pollen sources. However, 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 then be expected to be lower than the year before, irrespective of treatment. Without reference to pollen counts and without



a



b

Fig. 6. Total Timothy N allergen dose (a) given during 2 years treatment, and symptom scores recorded after treatment as correlated to the initial allergen threshold dose (TD) and reduction in nasal patency as measured with PEFRn (b).

Table 5

The median values and range of total serum IgE determined by PRIST and IgE antibodies determined by RAST. The *P*-values are given for the differences between the N- and O-groups within each year and within the groups between the years (*) respectively

Year	1975		1977	
Patient group	Timothy-N	Timothy-O	Timothy-N	Timothy-O
PRIST				
Median	375	156	264	197
Range	35–1,527	25–1,683	28–1,835	41–1,635
<i>P</i> -value	0.62		0.62	
<i>P</i> -value ^o	N: 0.44 O:0.46			
RAST				
Median	51.5	43.4	39.1	35.5
Range	6.6–60.6	10.4–56.4	5.1–56.0	13.8–62.4
<i>P</i> -value	0.14		0.84	
<i>P</i> -value ^o	N:<0.001 O:0.12			

objective assessment of the allergenic tolerance threshold this would easily have led to biased interpretations of the results of any treatment. Although use of symptom score and drug consumption diaries may have a limited reliability due to their subjective character, the results were confirmed by those of the objective assessment in this study.

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 absolutely identical allergen preparation before and after treatment is mandatory for this kind of evaluation. It was shown in this study that the allergenic activity of the refined allergen preparation was the same throughout the study period. Thus, the good reproducibility of the results achieved in the NPT performed in the control individuals indicates a good reproducibility of the NPT itself, confirming the results of challenges with histamine (5).

There is no standardized and generally accepted way to express the degree of nasal tissue reactivity to allergen challenge (13, 17). Parameters to be considered are concentration and dose of allergen introduced and degree of reaction to that particular dose. The site of application (17) and general status

and non-specific lability of the tissues may be critical (13). 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 as an indication of nasal tissue reactivity to the allergens seemed rational, although there may be several reservations concerning its use.

Decrease in skin sensitivity to intracutaneous tests after specific hyposensitization treatment has been reported (18). These reports do not, however, take into account the variability of allergenic activity of the extracts used and they could not be confirmed by our results. A positive skin test reaction to an allergen only indicates that IgE specific to that allergen is present in the skin. It does not necessarily indicate its role in the respiratory mucosa. Therefore, the allergenic response of the skin cannot be a true guide to the allergic state of a tissue so different in anatomical structure and physiological activity as the nasal mucosa.

In this paper only the direct quantitation of serum total IgE and grass pollen specific IgE antibodies have been included. Extended immunological studies will be published

elsewhere. After 2 years of treatment no statistically significant change in the total serum IgE was observed. IgE antibodies to timothy pollen as analysed by RAST showed a significant reduction in the N group but not in the O group. It is difficult to interpret these results. The reduction in serum IgE antibody concentration cannot solely explain the general clinical improvement and marked reduction in specific nasal sensitivity.

Our study demonstrated that prolonged, specific hyposensitization results in marked reduction of clinical symptoms and reduces nasal sensitivity to the allergens in question in the majority of patients. In fact, all patients in this study had some benefit from the treatment. Less favourable results have been reported by others. It is possible that the strict criteria used in our study to select patients for immunotherapy are partly responsible for the better results. Hyposensitization did not give an all-or-none effect, but resulted in a higher tolerance to the allergen in question.

Numerous investigations of hyposensitization therapy have suggested that the clinical effect seems related to the total dose of antigen given (16). 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 extracts. However, in our study the total allergen dose necessary to become effective appeared to depend on the initial degree of sensitivity, as suggested by the results of NPT with the well-defined allergen (Timothy N). The use of the crude extract rendered results which were less reliable for this comparison. The total doses of crude allergen extract necessary to give effects similar to that of the refined preparation, although showing marked variability, were up to three times higher for most patients. This could suggest that the somewhat better results obtained with the refined preparation may be due to a more favourable antigen competition situation than that obtained

with comparable total doses of the crude preparation.

This study demonstrates that the same or somewhat better results are achieved with a purified, representative and stable allergen preparation than with a crude extract of the same source. Refined, stable and well-characterized allergen preparations increase the safety of this treatment, rendering it possible for the physician to know with exactness the qualities of the material in use, and are preferable to crude extracts for many reasons (1).

Subsequent papers will report the results of more prolonged immunotherapy with consideration also of the effects on the nasal sensitivity to pollen from other grasses.

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