

Immunotherapy in Hay Fever with Two Major Allergens 19, 25 and Partially Purified Extract of Timothy Grass Pollen

A Controlled Double Blind Study. *In Vivo* Variables, Season I

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Forty grass pollen hay fever patients were randomly divided into two equal groups and treated from January 1978 with different timothy grass pollen extracts: whole pollen allergens (WPA) (Alutard® SQ), a partially purified extract and purified pollen allergens (PPA) consisting of the major allergens 19, 25. The effect was evaluated by symptom scores in grass pollen season I, 1978, and titrated thresholds of skin prick test (SPT) and nasal provocation test (NPT) with extracts WPA, PPA, and FGM (five-grass mixture).

Extracts were biologically standardized in the HEP (histamine equivalent prick) system (1 HEP=1,000 biological units (BU)/ml) and used in concentrations of 0.01–0.1–1.0–10 HEP for tests and treatment. The extracts for treatment were attached to Al (OH)₃. Extracts from the same batch were used throughout the investigation.

The two patient groups, WPA and PPA, were comparable as regards age, sex, total dosage (range 2,187–73,887 BU) and frequency of side effects. Fewer symptoms ($P=0.001$) were observed with WPA than with PPA. Titrated threshold increase showed a treatment efficacy of 70–100% in SPT compared with 15–65% in NPT and tended to show higher protection after treatment with WPA than PPA but equal protection against WPA and FGM, in accordance with common allergens in various grass pollen. In SPT with WPA the threshold increase was correlated ($P<0.05$) to symptom scores.

Frequency of major (urticaria, angioedema, asthma) general side effects was positively correlated ($P\leq 0.05$) to pretreatment NPT thresholds below 1 HEP and standard dosage at 1 HEP or more, but tended to a negative correlation with local side effects.

All local and general side effects first appeared 1–5 h after injection and a more careful dosage pattern than standard is recommendable at maximum concentration, 10 HEP, and also with extracts 1 HEP at pretreatment NPT thresholds below 1 HEP.

Key words: allergens; biological units; grass pollen allergy; hay fever; immunotherapy; major allergens.

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Specific immunotherapy (hyposensitization) is a well-accepted treatment for pollen allergic patients, but complete freedom from symptoms is seldom. Ragweed hyposensitization in particular has been used as a model in

controlled clinical and immunological trials, but treatment with grass pollen has given equal results (17, 23, 25, 26, 30).

No certain objective *in vivo* or *in vitro* parameters exist for a prospective evaluation of

treatment efficacy or selection of patients, but clinical improvement has been associated with a higher threshold of skin prick reactions (5, 29, 31) and nasal provocation tests (7, 8, 11).

To obtain better clinical results and reproducible analyses much effort has been expended regarding quality of allergen extracts, characterization, and purification.

Frankland & Augustin (9) presented the first controlled trial comparing treatment with crude extract with partially purified extract. Both extracts gave equal relief from symptoms. Recently, Frostad et al. (11), in a controlled study, used a crude extract, timothy O (TO), versus a partially purified extract, timothy N (TN); the latter approximately equal to WPA (whole pollen allergens) in this study. Groups TN and TO had clearly better results than a non-hyposensitized control group, but TN was considered better than TO.

Major allergens are the principal allergens of crude allergens. Previously, only one controlled clinical trial has been published comparing crude allergen with major allergen, the crude ragweed allergen and the major ragweed allergen, antigen E (26). The study demonstrated no difference between crude allergen and major allergen, but both allergens were better than placebo.

Recently, the major allergens 19, 25 have been isolated from timothy grass pollen (19). RAST inhibition showed the inhibitory effect of antigens 19, 25 to be approximately 90% of whole timothy extract (18, 19).

The purpose of this trial was to correlate the clinical and immunological variables when hyposensitizing with partially purified timothy extract, whole pollen allergens (WPA) versus purified pollen extract (PPA) with the major timothy allergens 19, 25.

The study is a prospective, controlled double blind study, covering perennial treatment for 3 years.

Clinical results from season I, 1978, are presented here. Extensive, immunological studies will be published elsewhere (28).

MATERIAL AND METHOD

Design

Forty patients were randomly divided into two equal groups. Twenty patients were hyposensitized with WPA and 20 with PPA. The clinical effect was evaluated by symptom scores in the grass pollen season and threshold increase in a quantitative nasal provocation test before treatment and after 8 and 12 (at season) weeks' treatment, and also in a quantitative skin prick test before treatment and at season. At the same time *in vitro* variables were controlled: total IgE (PRIST), total activity of specific IgE (RAST), specific IgE versus individual allergens of the extract (CRIE), and blocking IgG antibodies in blood and secretion (28).

Patients

Forty patients, aged 15–43 years, with typical hay fever with or without asthma for more than two seasons participated in the investigation. In group WPA the median for duration of symptoms was 7½ years, range 3–21 years, in group PPA the median was 10 years, range 2–19 years. Hyposensitization with grass pollen extract is indicated when severe symptoms persist, despite previous symptomatic and prophylactic treatment. Before treatment, only grass pollen allergic symptoms were accepted. Patients previously hyposensitized were excluded together with patients suffering from chronic nasal disease, e.g. adenoids, or chronic lung disease, or other diseases affecting the general condition and ability to take part in the study. Pregnancy or planned pregnancy also implied exclusion. Written informed consent was obtained before treatment.

Allergen extracts

Extracts were produced and supplied by Allergologisk Laboratorium A/S, Copenhagen, January 1978. In the skin prick test (SPT) and nasal provocation test (NPT) three extracts were employed: two timothy

extracts, WPA (whole pollen allergens, Alutard[®] SQ), a partially purified extract consisting of approximately 20 out of the approximately 30 timothy antigens (20), PPA (purified pollen allergens) consisting (19) of the major allergens 19, 25, and a third extract, FGM, a five-grass mixture containing equal parts of *Dactylis glomerata* (orchard), *Holcus lanatus* (yorkshire fog), *Festuca pratensis* (meadow fescue), *Phleum pratense* (timothy), and *Lolium perenne* (rye grass). These are five of the most common grasses in Denmark, and are combined as in Allpyral five-grass mixture. The relative amounts of antigen 25 were 100, 100, 20 in WPA, PPA and FGM respectively as determined by quantitative immunoelectrophoresis. The relative amounts of protein were 100, 12, 100 in WPA, PPA and FGM respectively. In SPT, extracts in 50% glycerol were used and in NPT extracts were diluted in phenol saline (0.4% phenol/0.9% sodium chloride). Extracts for *in vitro* investigations were diluted in 0.9% sodium chloride. WPA and PPA, both alum-precipitated extracts, were used for immunotherapy. The extracts were biologically standardized in the HEP system: In at least 10 patients with moderate allergy 1 HEP showed a skin reaction for the allergen in question corresponding to a histamine reference, histamine chloride 5.43 mmol/l = 1 mg/ml (1 HEP = 1,000 biological units (BU)/ml). Extracts were used in concentrations of 0.01-0.1-1.0-10 HEP, both during testing and treatment. According to the producer all extracts were dialysed; molecules of less than 8,000 daltons were removed together with unspecific irritants. Extracts from the same batch were used throughout the investigation.

The patients were all tested between January 1978 and June 1978. Extracts for testing and immunotherapy were stored at 4°C.

Immunotherapy

Immunotherapy was carried out weekly from January 1978 until the season, June 1978,

following the planned doses as shown in Table 1, and effectuated by the author assisted by nurses. All local and general side effects were registered at each control. All patients were out-patients. The maximum maintenance dose was 10,000 BU or else the highest tolerated, i.e. the dose just below one relapse of major general side effects or four relapses of local reactions of more than 10 cm in diameter with the same dose. From grass pollen season 1978 the interval between the injections was increased to 4 weeks. In the grass pollen season the dose was reduced 1/3-1/4. After each injection the patients were under observation in the clinic for 30 min and the diameter of local reactions was measured. The patients registered local side effects at home measuring the largest diameter after 8 h, or at maximum reaction. Local skin itching was also registered together with general side effects. Injections were given subcutaneously on the dorsal surface of the forearm corresponding to the middle 1/3. The piston was retracted to avoid intravenous injections.

Symptom scores in grass pollen season

Diary cards were kept daily during the season. Fig. 1 demonstrates the score system for eye, nose, and lung symptoms, and use of medicine. Medicine was handed out freely; one spectre of medicine for all patients was attempted,

Table 1

Standard dose at weekly immunotherapy starting January 1978 using from 2-10,000 biological units (BU).

Week no.	Millilitres	HEP/ml	BU
1	0.2	0.01	2
2	0.5	0.01	5
3	0.1	0.1	10
4	0.2	0.1	20
5	0.5	0.1	50
6	0.1	1.0	100
7	0.2	1.0	200
8	0.5	1.0	500
9	0.1	10.0	1,000
10	0.2	10.0	2,000
11	0.5	10.0	5,000
12	1.0	10.0	10,000

0 = no symptoms 1 = mild symptoms 2 = moderate symptoms 3 = severe symptoms		Diary card for one week. Registration of daily symptoms according to this scale 									
DATE	NOSE			EYES		LUNGS		NUMBER OF:			
	block- ing	run- ning	sneez- ing	red- ness	itch- ing	cough- ing	wheez- ing	anti- histamine tablets	anti- histamine eyedrops	cortico- steroid tablets	asthma aerosol puffs

Fig. 1. Diary card for daily registered symptom scores and use of medicine. One spectre of medicine was used for all patients.

successfully. Concomitant medication prescribed was a single antihistaminic for treating eye and nose symptoms. If treatment was insufficient corticosteroid tablets were given, but at minimum dose. Patients with asthma received aerosol. Before breaking the code, the medicine used was given scores as follows: antihistaminic eyedrops, (Antistina-Privin[®] containing Antazolin sulfas 5‰—Naphazolini nitras 0.25‰) 1 drop=score 1; antihistamine tablets (Polaramin[®], 2mg, containing Dexchlorpheniramin maleas) 1 tablet=score 2; corticosteroid tablets (Prednisone, 5mg) 1 tablet=score 6; and salbutamol aerosol (Ventolin[®], 1 puff=0.10 mg) 1 puff=score 1. Daily scores for eye, nose, and lung symptoms + medicine were recorded weekly throughout the grass pollen season.

Skin prick test (SPT)

The skin prick test was performed (27) using WPA, PPA, and FGM parallelly in 10-fold increasing allergen concentrations from 0.01 to

10 HEP on the volar side of the forearm from approximately three fingers' breadth below the elbow flexure to a hand's breadth above the wrist. The prick in the skin was made with an ordinary blood lancet directed through the allergen drops at an angle of 60–70° to the skin, the lancet point only "catching" the skin and lifting it, thus making a canal through which the extract could penetrate intracutaneously. The lancet was then pulled out carefully, and after 30–60 sec the drops were wiped off. Histamine HCl 1 mg/ml (5.43 mmol/l) was used as a reference for recording the SPT results and repeated at every SPT titration. The urticaria reactions were read after 10–20 min. The contours of the weal were marked by a soft ball point pen and by means of tape transferred to squared millimetre paper. The skin reaction was recorded as half of the largest weal diameter + the perpendicular diameter, and for the allergen reactions in per cent of that of the histamine reference (1). The threshold was expressed as the minimum allergen concentration giving a histamine equivalent urticaria (75% or more).

Nasal provocation test (NPT)

Like the other tests in this study, NPT was carried out on out-patients tested weekly with one of the allergens WPA, PPA, or FGM in increasing concentrations by factor 10 from 0.01 HEP to 10 HEP, or positive reaction. Simultaneous registration of three parameters (24) was used in NPT. Positive NPT was defined by two of the three following parameters: ≥ 0.5 ml secretion, $\geq$ five sneezes, $\geq 20\%$ decrease in nasal peak flow. All medicine was suspended for at least 3 days prior to the test. In NPT the patients inspired to maximum, holding their breath for some seconds, and by means of a DeVilbiss® atomizer No. 15, 0.2 ml allergen extract was atomized into the nose, three puffs into each nostril. The patients expired through the mouth to avoid aspiration of the allergen extract. Phenol saline was used as a negative control before allergen provocations. The reaction was registered after 10 min and if the reaction was negative a provocation with a higher allergen concentration was initiated. NPT often lasted about 1 h for each allergen and the patients were kept under observation in the clinic for approximately 1 h after the last provocation. Just after provocation each patient bent his head forward for 10 min and nasal secretion was collected in a funnel equipped with a syringe in which the volume could be measured directly. Peak flow measurement was recorded with a Wright® Peak Flow Meter equipped with a special mask, and the highest value of three peak flow measurements was recorded before provocation and before increase in allergen dose. The threshold was expressed as the lowest allergen concentration causing positive NPT.

Statistics

Unpaired Rank Sum Tests, Mann-Whitney or Kruskal-Wallis, Spearman's correlation analysis and Fourfold Table Test were

employed. Data were computerized in Rigshospitalet's own data department.

Pollen counts

Pollen grains were collected in a Burkard Recording Volumetric Spore Trap placed in the centre of Copenhagen, 15 m above the ground. Determinations and counting were carried out by the Aerobiological Group in Copenhagen and published in the Danish Pollen Bulletin (33). The weekly accumulated pollen counts were used. All the patients were living within a radius of 25 km of the spore trap.

RESULTS

The code for treatment and test extracts was not broken until after registration of all results and elaboration of the planning. The staff who would be testing the patients remained blinded to the code. All patients completed the investigation. Groups WPA and PPA were comparable in relation to age, sex, and also associated asthma. Fourteen men and six women received extract WPA and 12 men and eight women extract PPA. Mean age for either groups and sexes was 24 years, range 15–43 years in group WPA and range 15–38 years in group PPA. Five patients in group WPA and five in group PPA had associated asthma throughout the grass pollen season. One patient in each group had debut of asthma but three in group WPA with pretreated pollen asthma had no asthma in this season.

Dosage

Total dosage in the two groups was fully comparable. Fig. 2 illustrates medians and range for accumulated dosage after 8 and 12 weeks (at season) respectively.

After 8 weeks the median dosage in group WPA was 9,878 BU, range 2,500–18,887 BU, versus 10,887 BU, range 481–18,887 BU in group PPA.

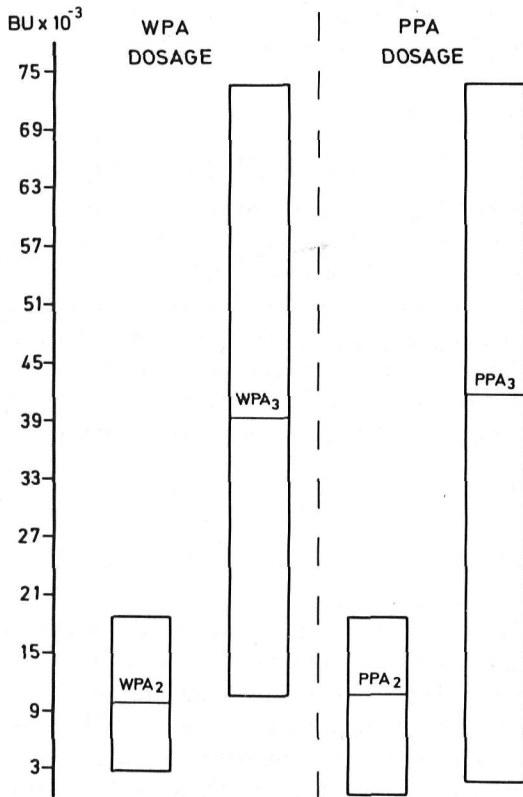


Fig. 2. Medians and range for accumulated dosage after 8 and 12 weeks, respectively. Total dosages in the two groups, WPA and PPA, were fully comparable.

After 12 weeks (at season) the median dosage in group WPA was 39,387 BU, range 10,957–73,887 BU, versus 41,887 BU, range 2,187–73,887 BU in group PPA.

At maintenance dosage the median in group WPA was 7,000 BU as single dose, range 3,000–10,000 BU, versus 5,500 BU, range 200–10,000 BU in group PPA. In group PPA four patients received $\leq 1,000$ BU, the rest $\geq 3,000$ BU.

Symptom scores

Fig. 3 shows the total scores, accumulated weekly for all 40 patients, week numbers as abscissa and total scores as ordinate. The weekly accumulated pollen counts/ m^3 ($\times 1/2$) for both birch and grass pollen are for practical purposes also shown on the same numerical

ordinate. The slight peak on the two curves for weeks 17–22 corresponds to début of symptoms in two birch pollen allergic patients in group WPA and three in group PPA; the dotted lines illustrate the results for weeks 17–22 when excluding the birch pollen allergic patients. The grass pollen season was most severe in weeks 23–25. The curves for WPA and PPA follow pollen counts and coincide after week 28, but up to week 28 there was a significant difference. Patients receiving WPA extract had significantly less symptoms than those receiving PPA.

Figs. 4–7 illustrate the selective eye, nose, and lung symptoms, and medicine scores. As expected, also here, a significant difference was seen between treatment with WPA and PPA, especially as regards medicine scores, whereas for lung symptoms there was none.

In Table 2 the *P* values illustrate the difference between the effect of WPA and PPA as regards the selective symptoms and use of medicine.

Generally, patients in group WPA report good or very good effect compared with moderate effect in group PPA. Therefore, median scores 113 for group WPA was chosen as the limit for mild/moderate symptoms and in group PPA median 218 as the limit for moderate/severe symptoms.

Six patients in group WPA versus two in group PPA were symptom free. Symptom free and mild (score 0–113), moderate (score 114–218), and severe (> 218 scores) symptoms were detected in 10 (vs. 5), 7 (vs. 5), 3 (vs. 10) patients in group WPA (vs. PPA).

Conjunctivitis scores paralleled rhinitis scores in most patients except three in group WPA who all had moderate (score 114–218) symptom scores without medication but no eye symptoms, only pretreated conjunctivitis. The season, too, lasted longer for patients in group PPA. In week 22 or before, five patients in group WPA showed symptoms compared with nine patients in group PPA. Correspondingly, in week 33 or after, one patient in group WPA showed symptoms compared with four patients in group PPA. No correlation existed between

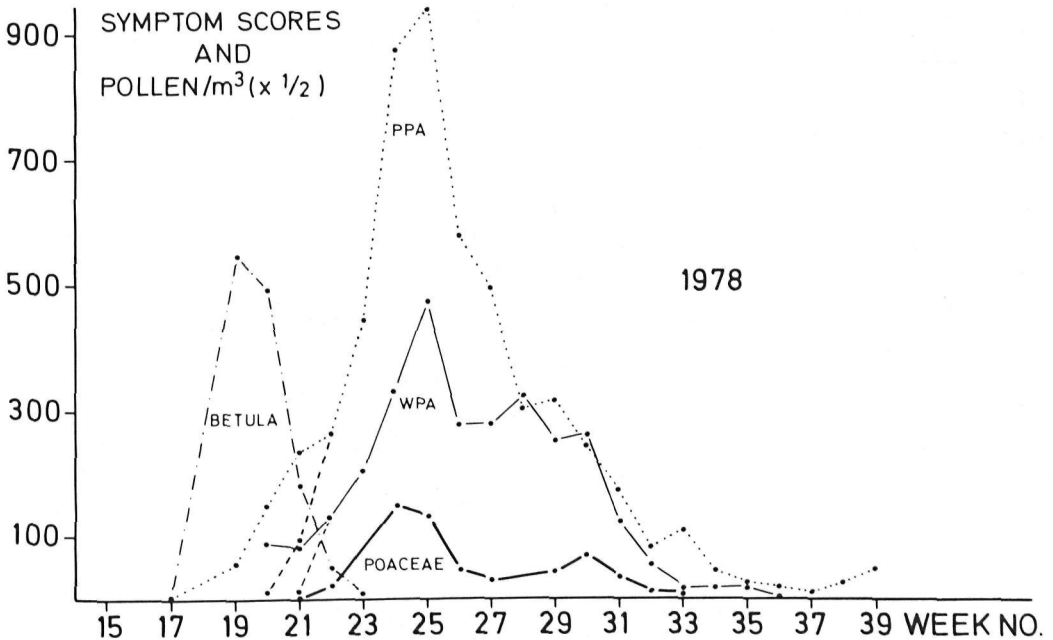


Fig. 3. Weekly accumulated scores for all 40 patients. Week numbers (abscissa). Total scores and weekly accumulated pollen counts/ m^3 ($\times 1/2$) for both birch and grass pollen (ordinate). The slight peak on the two curves for weeks 17–22 corresponds to the birch pollen allergic patients in each group; the dotted lines illustrate the results for weeks 17–22 when eliminating the birch pollen allergic patients. Symptom scores followed pollen counts, and group WPA had significantly less symptoms than group PPA.

accumulated dose and total scores, or the most significant "symptom", medicine scores.

Threshold increase in SPT and NPT

Figs. 8–10 illustrate the distribution of threshold levels in groups WPA and PPA, before treatment and at season in NPT and SPT with the extracts WPA, PPA, and FGM.

Increase in SPT thresholds (Table 3) for extracts WPA, PPA, and FGM was shown in 85–100% of group WPA compared with 70–75% of group PPA. The tendency to receive better protection from WPA (100%) than PPA (75%) treatment was seen in SPT with PPA. In SPT with WPA the threshold increase was correlated ($P < 0.05$) to symptom scores (Table 4).

Increase in NPT thresholds for extract PPA was not comparable with the two other

allergens because of high pretreatment thresholds; four patients in group WPA and six in group PPA had negative NPT for PPA (Fig. 9). In NPT WPA treatment (65%) tended to give better protection than PPA (35%) against FGM, and more (40%) than PPA (15%) against PPA (Table 3). NPT threshold increase for WPA was equal at treatment with WPA (50%) and PPA (45%) but NPT with WPA tended (Table 4) to higher threshold increase (8/40 patients) than NPT with FGM (4/40 patients). No correlation for symptom scores and NPT threshold increase was detected (Table 4).

WPA treatment demonstrated no significant difference between protection in SPT/NPT against WPA and FGM.

Threshold increase in NPT and SPT with WPA, PPA, and FGM was not correlated to dosage, neither accumulated dose after 8 weeks, nor after 12 weeks (at season).

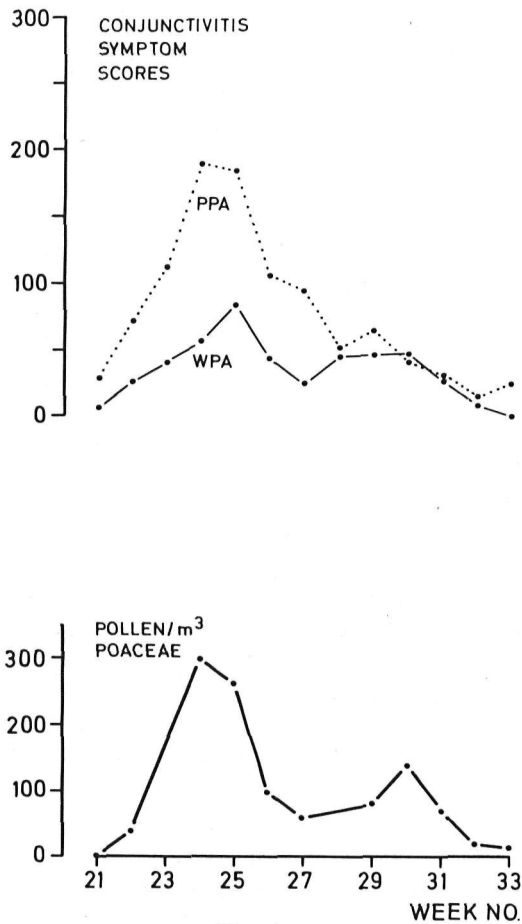


Fig. 4.

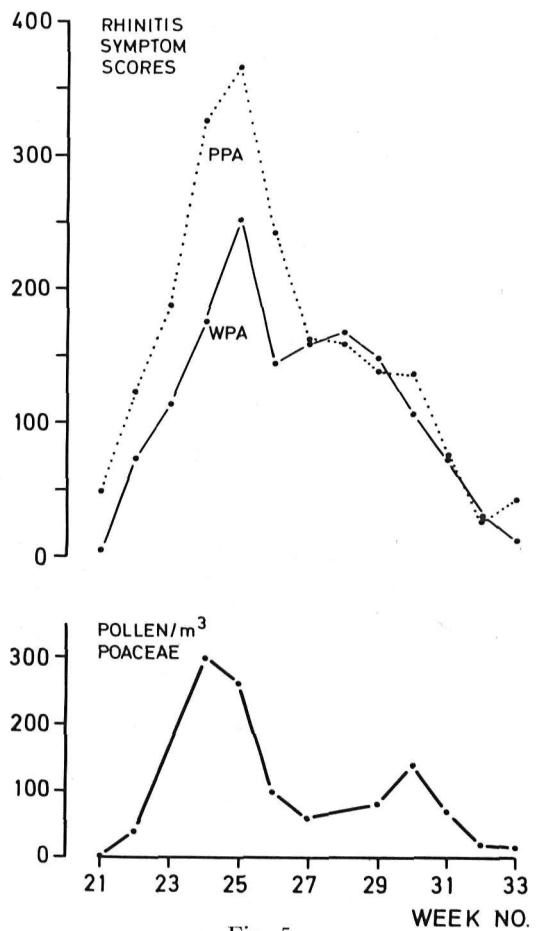


Fig. 5.

Reproduction of dosage versus SPT

In groups WPA + PPA until season 1978 a reproduction of the same dosage level had been carried out 50 times, partly due to maintenance dosage, and partly to local reactions and minor general reactions, especially tiredness, and in connection with preceding nasal provocation test. By repeating the dose once the mean reduction of skin reactions was 49% in group WPA and 40% in group PPA, with no significant difference whether the reactions were more or less than 10cm.

Side effects with immunotherapy

Local side effects. Table 5 illustrates 25%, 50% (medians), and 75% percentiles of skin

reactions versus number of patients and standard dosage. Generally, adult patients tolerate local reactions below 10cm—the diameter limit chosen for local side effects—and the dosage pattern was changed only when skin reactions were more than 10cm, or if there were general side effects. On the whole, the two groups reacted equally, although in group PPA the patients tended towards a stronger allergic reaction at the lowest and highest dosage. Due to local and general side effects only approximately half of the patients reached 2,000 BU at standard dosage.

In group PPA only 8/20 patients followed the standard dosage, but two had local reactions of more than 10cm at maximum dosage. In group WPA 5/20 followed the standard dosage right up to maximum dosage,

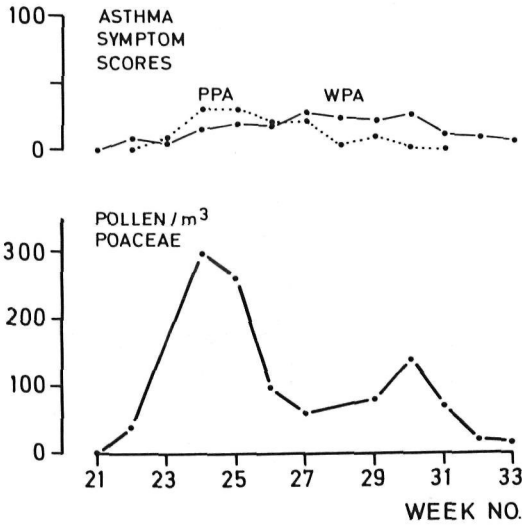


Fig. 6.

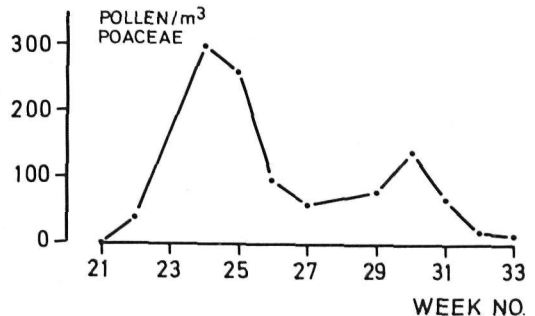
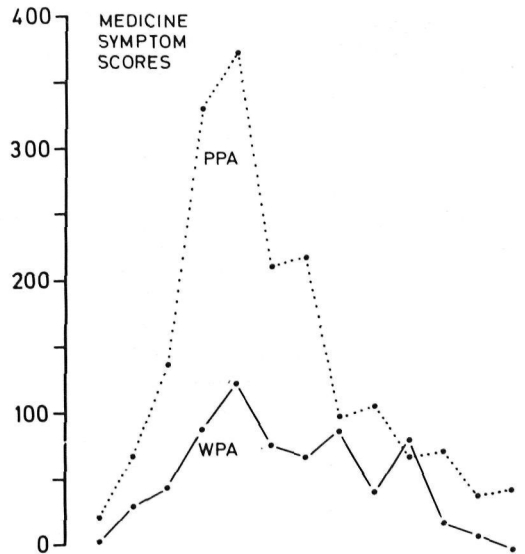


Fig. 7.

Figs. 4–7. The selective eye (Fig. 4), nose (Fig. 5), and lung (Fig. 6) symptoms, and medicine scores (Fig. 7). Significant difference is shown between groups WPA and PPA, especially as regards medicine scores, whereas for lung symptoms there is none. Excluding group WPA lung symptoms, the scores follow pollen counts. (For *P* values, see Table 2).

but one had urticaria and two had local skin reactions of more than 10 cm.

Dosage levels at appearance of the first local side effects in the two groups, 14 patients in each group, are shown in Fig. 11. At low and high doses the PPA group showed slightly more local side effects than the WPA group. Only a few patients showed the first heavy local reaction at single dose above 1,000 BU. The frequency of all general side effects was correlated neither to frequency of local reactions nor dosage level at appearance of local reactions, but major general side effects tended towards negative correlation to local side effects (see below).

When using aqueous extracts local side effects appeared within the initial time of observation, i.e. 30 min, whereas the alum-

precipitated extracts used in this study first showed local side effects after 1–2 h, generally 6–8 h at maximum, and most of them disappeared within 24 h. Twelve of the 28 local side effects appeared at single dosage 500 and 1,000 BU. Most patients with significant local side effects had relapsing local side effects on later increase of dosage.

In both groups 19/20 had local skin itching, appearance after 1/2–2 h, mean duration 48 h, range 1/2–72 h.

General side effects. Fig. 11 (on the right) shows the general side effects and frequency of subcutaneous nodules in groups WPA and PPA. Twenty-two patients had 33 general side effects dispersed among only 10 symptoms. In group WPA eight patients showed 11 reactions

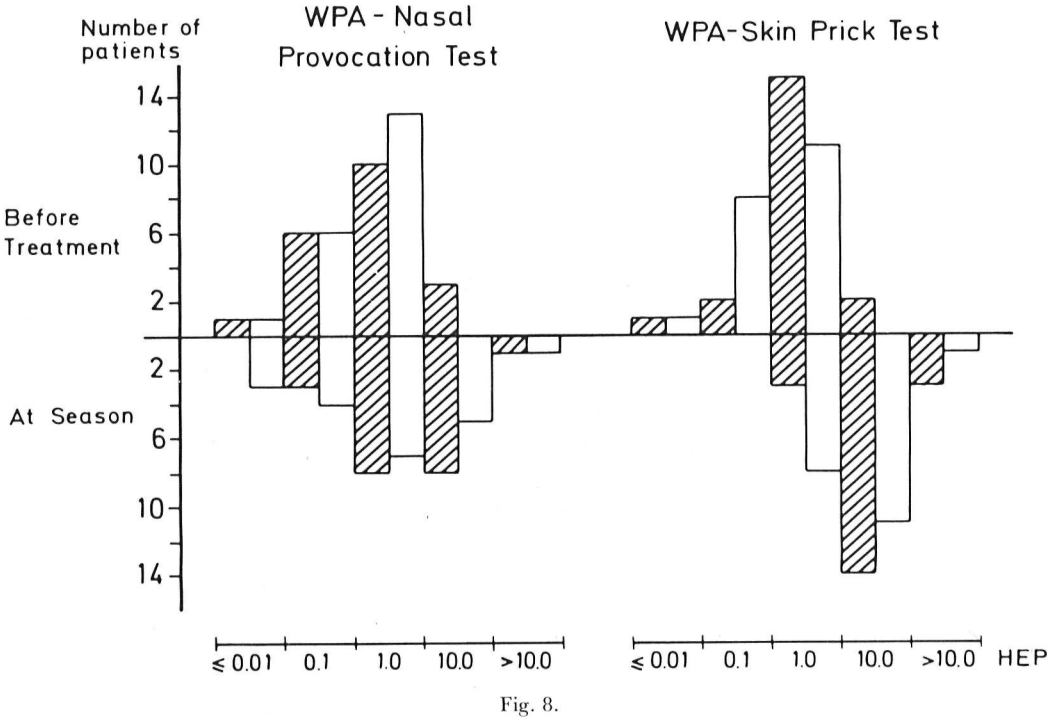


Fig. 8.

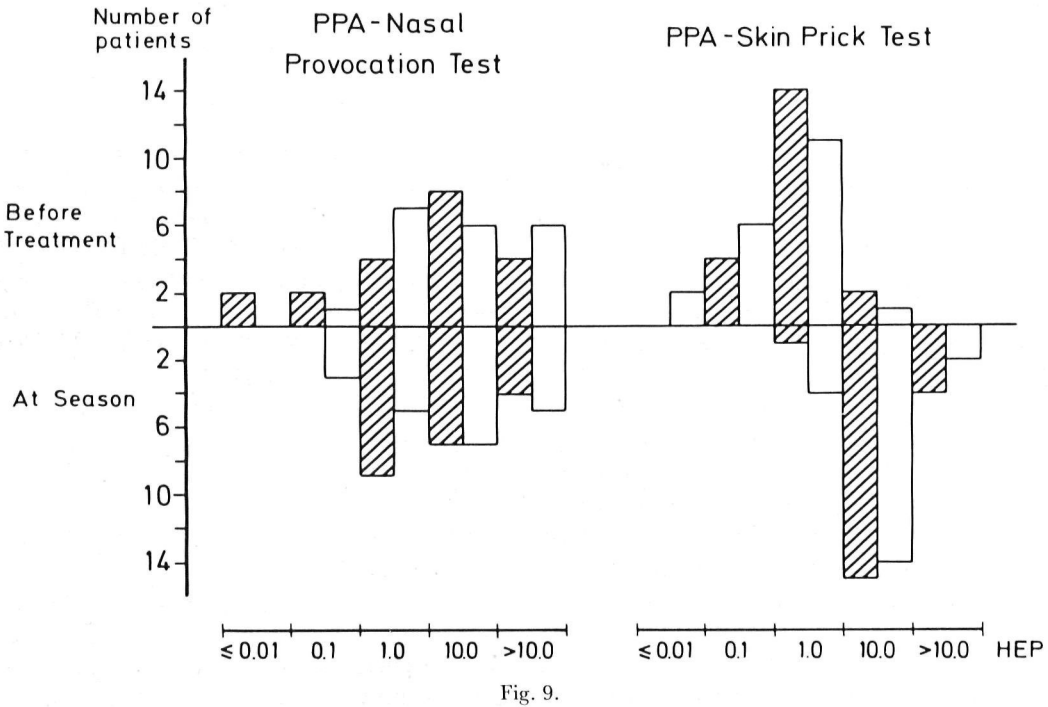
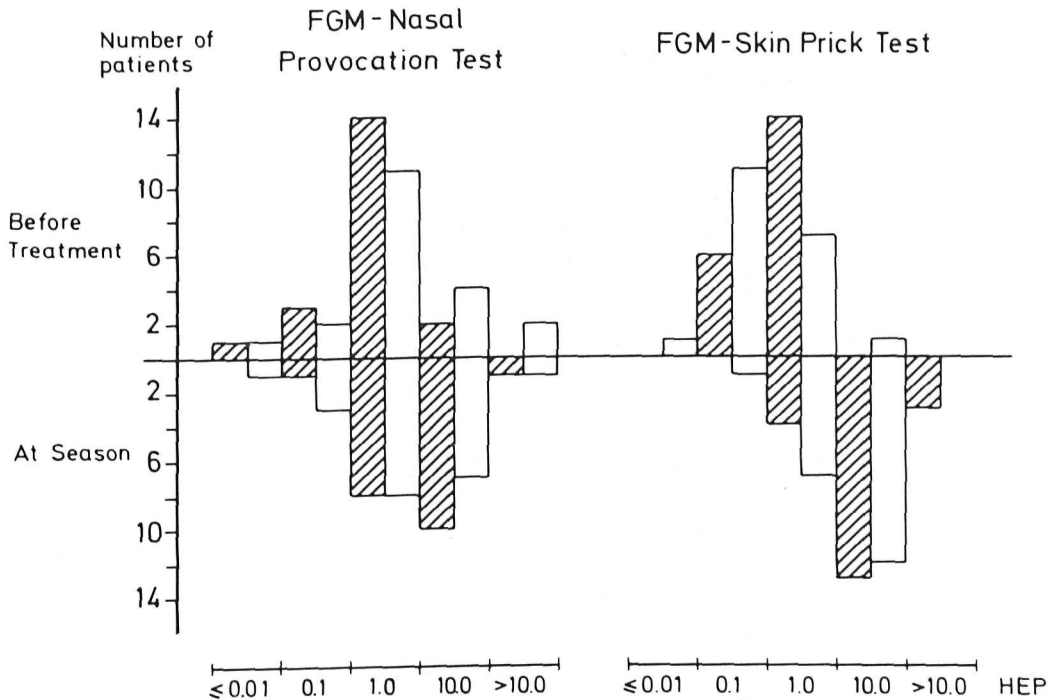


Fig. 9.



Figs. 8-10. Threshold levels in group WPA $\square$ versus group PPA $\square$ before treatment and at season in NPT and SPT with the extracts WPA, PPA, and FGM. The two groups were comparable according to pretreatment threshold levels. (For frequency of threshold increase after hyposensitization, see Tables 3 and 4).

and one of them had subcutaneous nodules. In group PPA 14 patients had 22 general reactions and, in this group too, one had subcutaneous nodules.

The major general side effects were urticaria, moderate angioedema, and asthma.

Table 2
P values for difference in symptom scores between group WPA and group PPA.

Difference in symptom scores	Group WPA versus PPA <i>P</i> values at weeks 23-25		
	23	24	25
Total	0.030*	0.001***	0.012*
Medicine	0.005**	0.004**	0.006**
Conjunctivitis	0.077	0.005**	0.005**
Rhinitis	0.057	0.014*	0.040*
Asthma	0.550	0.595	0.533

* $P < 0.05$

** $P < 0.01$

*** $P \leq 0.001$

As shown in Fig. 11, these reactions appeared in only one patient at standard dosage below 1,000 BU whereas in most of the patients the reactions appeared at the two dose levels 1,000 and 2,000 BU; in group WPA six patients recorded seven major reactions, in group PPA

Table 3

Number and percent () of patients with 10-fold threshold increase in SPT and NPT in the two groups treated with WPA and PPA for the three allergens WPA, PPA, and FGM.

No. and % () of patients with increase in SPT and NPT thresholds		SPT and NPT allergens		
		WPA	PPA	FGM
Group WPA	SPT	17(85)	20(100)	18(90)
	NPT	10(50)	8(40)	13(65)
Group PPA	SPT	14(70)	15(75)	15(75)
	NPT	9(45)	3(15)	7(35)

Table 4

Tenfold threshold increase in SPT and NPT versus total individual symptom scores. Significant increase in symptom scores with increase in SPT thresholds is shown when testing with WPA.

Tenfold threshold increase in SPT and NPT	Median symptom scores and no. patients () in SPT and NPT in groups WPA/PPA with the allergens:					
	WPA		PPA		FGM	
	SPT	NPT	SPT	NPT	SPT	NPT
≥ 2	119(13)	126(8)	103(13)	286(3)	94(13)	175(4)
1	125(18)	248(11)	156(22)	127(8)	167(20)	134(16)
≤ 0	298(9)	148(21)	248(5)	136(29)	205(7)	193(20)
<i>P</i> values	0.017*	0.325	0.272	0.539	0.250	0.532

**P* < 0.05

seven patients recorded nine major reactions. With both major general reactions and local side effects the dosage pattern was later changed in an attempt to increase the dosage by only 0.1 ml per dose. In two patients in group PPA this resulted in relapse at the same dose (angioedema then urticaria), and at one dose level higher (urticaria then asthma), and in group WPA relapse in one patient (asthma, then at two dose levels higher, urticaria). Three out of thirteen patients with heavy general reactions later reached the same dose level, and 6/13 reached higher dose levels without relapse, but in 7/13 patients higher dosage than at major general reactions was avoided due to maximum dosage (three patients), relapses of local (two patients), and general reactions (two patients). Unfortunately,

all general reactions first appeared after 1 1/2–5 h and lasted, without treatment, from 1/2–10 h, except for one patient who had urticaria for one entire week (No. 23), the reactions appearing after 6–8 h. Due to general reactions, one patient was admitted to observation for some hours, a few patients contacted their respective general practitioners and received antihistamine with good effect. As regards the rest of the patients, their symptoms disappeared spontaneously. In addition to the side effects, as shown in Fig. 11, patient No. 35 lost considerable weight, approximately 10 kg, but because of several side effects this patient's immunotherapy was suspended September 1978. The patient had received the lowest dosage, only 2,187 BU at season, but showed more symptoms than the

Table 5

Local skin reactions, 25%, 50% (medians), and 75% percentiles, versus number of patients and standard dosage. Only about half of the patients reach 2,000 BU or more at standard dosage. (For details, see text).

No. patients in group	WPA	20	20	20	20	20	19	19	19	17	11	9	5
	PPA	20	19	19	19	18	18	18	16	16	12	10	8
Local skin reactions in cm													
75% percentiles	WPA	2	8	7	7	10	10	10	14	13	12	12	13
	PPA	8	8	7	10	6	10	10	11	12	9	10	15
50% (medians) percentiles	WPA	0	2	4	3	4	7	7	5	9	8	8	8
	PPA	2	3	2	5	4	5	6	9	7	6	7	13
25% percentiles	WPA	0	0	0	0	0	0	4	0	7	3	5	4
	PPA	0	0	0	1	0	2	3	4	5	5	4	9
Standard dosage	BU	2	5	10	20	50	100	200	500	1,000	2,000	5,000	10,000

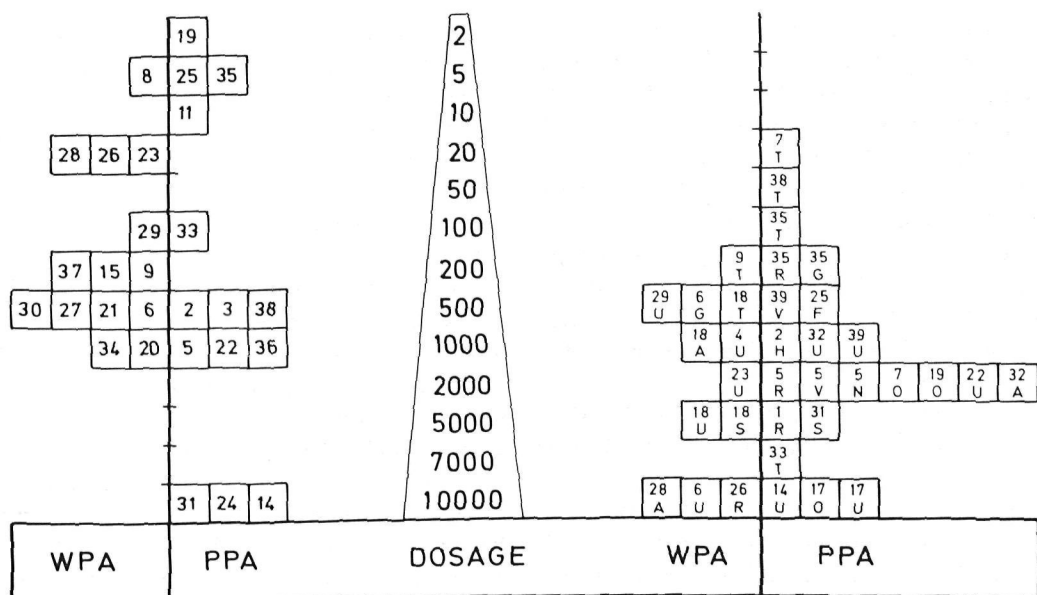


Fig. 11. General (on the right) and local (on the left) side effects in the two groups, WPA and PPA. Comparing the local and general side effects with the frequency of side effects in the same patients the boxes are marked with the code number for each patient. *Minor general side effects:* tiredness (T), rhinitis (R), arthralgia (G), conjunctivitis (V), flu-like symptoms (F), headache (H), nausea (N), and subcutaneous nodules (S). *Major general side effects:* urticaria (U), angioedema (O), and asthma (A) appeared in only one patient at standard dosage below 1,000 BU. Frequency of major general side effects was significantly correlated to pretreatment thresholds in NPT. The high frequency of local and general side effects was mostly caused by too fast an increase in standard dosage. (For details, see text).

other patients. It later appeared that this patient had considerable family problems which might explain both the loss of weight and some of the general symptoms. The patient was controlled clinically, including haematological and serological tests, but no other pathological signs were revealed. He gained normal weight shortly after stabilization in the family 4–6 months after the last immunotherapy.

Pretreatment SPT thresholds were not correlated to dosage level or frequencies of local and general side effects, but frequency of major general reactions was significantly correlated ($P \leq 0.05$) to standard dosage at $\geq 1,000$ BU, and pretreatment NPT thresholds; 8/13 patients with major general reactions had pretreatment NPT thresholds for WPA below 1 HEP compared with 6/27 patients without major general reactions.

Most of the patients, 28/40, developed local side effects. Seven out of 28 patients with local

side effects showed major general reactions at higher dosage compared with 6/12 patients without local side effects, the patients with relapsing general reactions (Nos. 17, 18, 32) being in the latter group, thus showing a tendency to a negative correlation between local and major general reactions.

Neither local nor general reactions were correlated to symptom scores.

DISCUSSION

Groups WPA and PPA were fully comparable in age, sex, associated asthma, dosage, and, in general, in basic values of serological parameters (28). Basic threshold values of NPT and SPT of allergens WPA, PPA, and FGM were equally distributed between the groups.

However, significant differences in the effect of WPA and PPA were recorded in high

season, in total score, and also in the individual symptoms. The number of patients being insufficient, a clear comparison of lung symptoms was not possible, but at the beginning of the grass pollen season, and during the high season, most PPA scores were recorded, and at the end most WPA asthma scores were found, but only PPA scores seemed to follow pollen counts. No peak for scores in relation to the high season for mould fungi, weeks 30–34, was reported, as has been demonstrated by others (4). Asthmatic patients showed the same results as the other patients as regards SPT/NPT thresholds, side effects, and dose levels.

Difference between the groups was most significant in medicine scores (use of medicine) partly equalizing symptoms between the groups. Patients were controlled regularly throughout the season, and carefully filled in their daily score registration cards.

Evidently the partially purified extract, WPA, with approximately 20 of 30 timothy antigens and containing all the allergens (19, 32) proved in this first season significantly more effective than extract PPA with major allergens 19, 25, although *in vitro* these allergens inhibited approximately 90% of the allergen activity of timothy in RAST inhibition (18, 19).

Recently, a controlled study (11) compared the effect of the refined extract (12), timothy N (TN), with timothy O (TO), a crude extract. Like the allergens, WPA, PPA and FGM in this trial, TN and TO were biologically standardized in the HEP system and TN, the refined extract, almost corresponded to WPA (20), but with a very low content of antigen 30. The effect of TN and TO was demonstrated in relation to a non-hyposensitized unblinded group; no placebo was used, but the refined extract, TN, was considered better than the crude extract, TO.

This investigation with a maximally purified extract, PPA, had shown in the first season that extracts can be too purified. Furthermore, group PPA showed the same frequency of side effects as group WPA and tolerated a slightly

lower dosage than group WPA. Perhaps additional treatment with the allergens 15 and 30 (28), or long-term treatment with PPA, could equalize WPA.

Why do only few in group WPA, as in other trials hyposensitizing hay fever patients, become symptom free?

Inspired by Morrow Brown (3), patients were treated weekly with much higher dosages than standard until the season started, but the problems do not seem to be solved by high dosage only. WPA treatment demonstrated no significant difference between protection against WPA and FGM in agreement with the pronounced cross identity of ordinary grasses, but tendency to a higher threshold increase in NPT with WPA than with FGM, and the correlation between SPT decrease and symptom scores for WPA only could indicate the need for some specific allergens in grass pollen treatment extracts, or individual therapy. *In vivo* and *in vitro* (2, 6, 16, 18, 22) investigations have demonstrated 10–20% specific allergens in common grasses, but the lack of more or less specific allergens in the treatment extracts could give much more than 10–20% of the symptoms and could be the reason why there is seldom complete freedom from symptoms after immunotherapy.

It is not clear why WPA treatment tended to give better protection against PPA provocation than PPA treatment; the immunological studies have confirmed this tendency (28). Perhaps PPA antigens are more effective together with other timothy antigens, as in WPA, causing an enhanced antigen effect.

Previously, investigators have discussed the effect of hyposensitization correlated to skin sensitivity. Some could reproduce a correlation (5, 29, 31) whilst others lacked proof (11, 21, 26). In this study a significant correlation was demonstrated between thresholds in SPT with WPA and symptom scores. The exactly titrated threshold at 10-fold level was needed. All skin reactions with extracts 1 HEP were estimated, but neither absolute nor relative decreases in the skin reactions with or without correction for

histamine reference were significantly correlated to symptom scores.

Only a few previous studies (7, 8, 11) have quantified NPT in hyposensitization, generally finding higher thresholds after hyposensitization, but not correlated to symptom score levels, just as in this study, season I. This could be due to too crude a method and/or a non-relevant, fixated demand for a clear reaction in 2 of 3 variables, but no correlation was demonstrated between symptom scores and change in number of sneezes, or nasal secretion, or decrease in peak flow when testing with all three extracts.

Pretreatment NPT thresholds did not correlate to symptom scores or accumulated dosage but to frequency of major general side effects.

Monitoring the dosage in hyposensitization until the NPT was negative, Morrow Brown (3) found 94% responders compared with 27% responders receiving conventional dosage in an open study on Allpyral five-grass mixture. This present trial could not reproduce these results; the reason could be that in NPT Morrow Brown employed an original microspoon for the dosage of dry pollen which corresponded to normal exposition.

Unlike some investigations (10, 14, 15), this study did not correlate dosage to symptom scores but a high dosage pattern was applied through weekly injections at the maximum tolerated dose in all the patients; thus dosage was adjusted to side effects. Despite a total dosage at season far above standard dosage only a few patients had negative NPT at season I, 1978.

SPT and NPT parameters also indicate the effect of PPA extract, though they depend on results of the following seasons. It is not possible to state whether PPA extract had a satisfactory effect as no placebo group was included. This investigation supports other studies on the effectiveness of hyposensitization with grass pollen extracts—as early as in the first season when using the high dosage pattern—but only if PPA extract is as good or

better than placebo.

Side effects have not often been described in previous publications (30). In this study the frequency of both local and general reactions was relatively high but equal with both extracts. The general reactions were partly caused by too fast an increase in standard dosage at the two highest concentrations, especially in patients with pretreatment NPT thresholds below 1 HEP. By altering the dosage pattern with an increase of 0.1 ml per injection after general reactions most patients reached the same dose level, or even higher, without relapse. In group WPA all patients reached maintenance dose at 10 HEP concentration, median 7,000 BU, in group PPA all except three, median 5,500 BU. No general reactions appeared in the grass pollen season. Most of the patients, 28/40, had local side effects due to the high dosage principle.

The dosage principle used in this study made it much easier to estimate limits for dosage than when using the low dosage principle.

With alum-precipitated extracts both local and general side effects were delayed. Alum-precipitated extracts therefore, unlike aqueous extracts, do not reveal local reactions immediately in relation to treatment, but the appearance of major general reactions in patients who have already left the clinic gives greater problems.

A more careful standard dosage is recommendable as regards the maximum concentration 10 HEP, i.e. a dosage pattern of 0.1-0.2-0.3-0.4-0.6-0.8-1.0 ml, but at pretreatment NPT thresholds below 1 HEP only an increase of 0.1 ml per injection with extracts 1 HEP and 10 HEP.

Alum-precipitated extracts in hyposensitization are by now in extension and require less injections than aqueous extracts at intervals of not less than 1 week. At maintenance dose an interval of 6 weeks $\pm$ 2 weeks is advisable.

Subcutaneous nodules appeared at the first season in two cases only with single standard dose starting at 5,000 BU. Guinea pigs, injected with alum-precipitated antigens but

not aqueous extracts, all generated nodules with characteristic cellular reactions (13). The consequence of these side effects is uncertain at the present stage of this study.

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