

Side Effects during Immunotherapy with Purified Grass Pollen Extracts

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In a 3-year prospective double blind study, grass pollen allergic patients were allocated to perennial hyposensitization with the timothy major allergens Nos. 19 and 25 (2-component extract) or a 20-component timothy extract. The extracts were biologically standardized and adsorbed to aluminium hydroxide for treatment.

Systemic side effects (SSE) had début after 1 ½-5 h and lasted without treatment 1/2-10 h. Treatment with the 2-component extract showed preponderance of minor SSE (arthralgia, rhinitis, tiredness, headache, conjunctivitis, nausea, flu-like symptoms), but major SSE (urticaria, angioedema, asthma) were equally distributed between treatment with the two timothy extracts.

Major SSE complicated the treatment before the first grass pollen season in 33% of the patients vs. only in 3% during the subsequent perennial therapy, and developed (92%) at high single dose of $\geq 1,000$ biological units. The majority (69%) were later able to reach the same or higher dose without relapse. Most (62%) patients with major SSE were predicted by high nasal sensitivity before treatment.

Only 18% of the patients had immediate local skin reactions of ≥ 2 cm, but delayed local side effects of ≥ 10 cm were recorded in 70%. Immediate skin reactions did not correlate with delayed skin reactions or with SSE, but delayed local side effects tended towards negative correlation with major SSE. A mean area reduction of 50% of the delayed skin reactions was recorded by repetition of a single dose.

Subcutaneous nodules appeared at single doses of $\geq 5,000$ biological units. Only 5% of the patients contracted nodules during initial preseasonal therapy compared with 38% during subsequent perennial dosage. The nodules contained typical benign granulomas, and the frequency in the two groups was proportionate to the quantity of aluminium in the two extracts.

Key words: allergens; biological units; granulomas; grass pollen allergy; immunotherapy; side effects.

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CLINICAL ASPECTS

Systemic and local side effects were delayed with aluminium adsorbed extracts. Hyposensitization with the timothy major allergens Nos. 19 and 25 (2-component extract) did not reduce the frequency of side effects compared to treatment with a 20 component timothy extract. Major systemic side effects were predicted by means of high nasal sensitivity but not by immediate local skin reactions and even tended towards negative correlation with delayed local side effects.

Abbreviations: SSE = systemic side effects; Major SSE = urticaria, angioedema, asthma; Minor SSE = arthralgia, rhinitis, tiredness, headache, conjunctivitis, nausea, flu-like symptoms; LS = local side effects; DLS = delayed local side effects; SN = subcutaneous nodules; MTD = maximal tolerated dosage (= maintenance dose); WPA = whole pollen allergens; a partially purified timothy extract consisting of

approx. 20 out of the extractable 30-45 timothy antigens, but all the potential allergens (20-component extract); PPA = purified pollen allergens: two isolated timothy major allergens, Ag 19 and Ag 25 (2-component extract); Group WPA = patients treated with WPA extract; Group PPA = patients treated with PPA extract.

It is essential to combine optimal efficacy of specific immunotherapy (hyposensitization) with minimal side effects.

The frequency of systemic side effects varies considerably in the literature but is influenced by many factors such as latency in side effects (7, 9, 12, 30, 31), dosage (5, 17, 28), method of incidence evaluation (18), type and purification of allergens (10, 11, 34), quality of allergen extracts (1), adjuvants (18, 28), tissue sensitivity (5), and actual errors (31, 34).

The purpose of this investigation was to evaluate the incidence and character of side effects during preseasonal and subsequent perennial treatment compared with other studies.

Employed in this study were equivalent doses of two biologically standardized grass pollen extracts of differing degrees of purification and varying contents of adsorbed aluminium hydroxide. The clinical efficacy during three grass pollen seasons is described elsewhere (19, 21).

MATERIAL AND METHOD

Patients

In a 3-year double blind study 40 typical grass pollen allergic patients were allocated to specific immunotherapy (hyposensitization) with WPA (group WPA), and PPA (group PPA), as described previously (19).

Allergen extracts

Extracts were produced and supplied by ALK, Copenhagen, Denmark, January 1978. Group WPA was treated with WPA (Alutard® SQ), a partially purified extract from timothy grass pollen (20-component extract). Group PPA was treated with PPA consisting of two isolated timothy major allergens, Ag 19 and Ag 25 (2-component extract) (13). The extracts were biologically standardized. Extract WPA contained 4.0 mg aluminium hydroxide/ml diluent in 10 HEP and extract PPA contained 1.2 mg aluminium hydroxide/ml diluent in 10 HEP. The stability of the extracts has been described in another publication (22). The amounts of

protein, and of Ag 19 and Ag 25 in extract WPA (1 HEP) were approximately 40, 3, and 6 µg/ml, respectively. The amounts of protein, and of Ag 19 and Ag 25 in extract PPA (1 HEP) were approximately 9, 3, and 6 µg/ml, respectively. The Nordic Committee for Allergen Standardization has defined 1 HEP extract as 1,000 biological units (BU)/ml (1). Extracts from the same batch were used for treatment and for *in vivo* and *in vitro* tests throughout the investigation.

Immunotherapy (hyposensitization)

Dosage has been described in detail, and maximal tolerated dosage (MTD) was used as maintenance dose (19, 21). All patients received one injection per week before season 1 (preseasonal therapy), and up to MTD just after seasons 1 and 2; otherwise one injection per month (subsequent perennial treatment). Seasonal dosage was reduced to 1/3–1/4. Injections were given subcutaneously, alternately into the left and right proximodorsal forearm. The final injections were given just before season 3 after approximately 116 weeks of hyposensitization.

Table 1

Number of systemic side effects (SSE) in group WPA and group PPA () before season 1 (preseasonal therapy).

Total SSE	11 (22)	100.0 %
Major SSE	7 (9)	48.5 %
Asthma	2 (1)	9.1 %
Urticaria	5 (5)	30.3 %
Angioedema	0 (3)	9.1 %
Minor SSE	4 (13)	51.5 %
Arthralgia	1 (1)	6.1 %
Rhinitis	1 (3)	12.1 %
Tiredness	2 (4)	18.2 %
Headache	0 (1)	3.0 %
Conjunctivitis	0 (2)	6.1 %
Nausea	0 (1)	3.0 %
Flu-like symptoms	0 (1)	3.0 %

Side effects

Systemic side effects (SSE) were divided into major and minor SSE according to Table 1.

After each injection the patients were observed in the clinic for 30 min and the diameter of local reactions was measured. At home the patients recorded the largest diameter of the delayed local reactions after 8 h or at maximum reaction. Generally, adult patients accepted delayed local reactions below 10 cm, the limit chosen for delayed local side effects (DLS).

Clinical effect

Symptom scores and concomitant medication were recorded daily throughout the pollen seasons. The score system has been published previously (19).

Effect parameters

Quantitated skin prick tests (SPT) and nasal provocation tests (NPT) were performed with 10-fold diluted allergen extracts from 0.01 to 10 HEP or until positive NPT. The threshold dose for SPT (skin threshold) produced reaction identical to histamine chloride 5.43 mmol/l or for NPT positive reaction (nasal threshold) (23). Total serum-specific and nasal-specific IgE (Al-RAST) and serum-specific IgE against individual timothy allergens (CRIE) as well as serum-specific IgG and anti-IgG were measured as published elsewhere (20, 22, 24).

Statistics

Paired and unpaired Rank Sum Tests and Fourfold Table Test were employed, using two-tailed tests with 5% significance level.

RESULTS

Systemic side effects

Systemic side effects during *preseasonal therapy* before the first grass pollen season have been presented previously (19). SSE had début after 1½–5 h and lasted without treatment 1/2–10 h,

except for one patient who had urticaria for one entire week.

The number and type of major and minor SSE are shown in Table 1. There was no statistical difference between groups WPA and PPA in the frequency of major SSE. Most patients had urticaria. Half of the SSE were minor, but 77% of the minor SSE occurred in patients treated with the two major allergens (PPA). In groups WPA (PPA) six (seven) and four (nine) patients had major respectively minor SSE.

One patient with angioedema and urticaria was admitted for a couple of hours and few patients consulted their respective general practitioners for treatment with antihistamines.

If major SSE occurred, dosage was reduced to 10–20%. A later careful increase in dosage by 0.1 ml per injection resulted in relapses in three patients, in one patient at the same dosage and in two patients at higher dosage levels. However, 69% (9/13) of the patients with major SSE reached the same dosage level or a higher level without relapses. No patients dropped out of the investigation because of major SSE. One patient with minor SSE and uncharacteristic weight loss left shortly after season 1. He regained his normal weight shortly after his family situation had stabilized 4–6 months after the last allergen injection.

During *subsequent perennial therapy* only one patient developed SSE (relapse of asthma) 6–8 h after one injection, but the symptoms disappeared after treatment with an aerosol. No other major SSE and only a few minor SSE were recorded in relation to MTD. One patient felt

Table 2

Median diameters (cm) of delayed local skin reactions (25–75% percentiles) during maximal tolerated dosage (MTD) in group WPA and group PPA just before the three seasons. Significant difference between the groups in 1978 was shown at 5% level

	Group WPA	Group PPA
1978	5 (5–10)	4 (0–5)
1979	9 (4–10)	6 (4–8)
1980	5 (4–10)	6 (3–7)

tired after MTD earlier the same day, and some injections caused slight axillary adenitis in two patients. Two patients had more unusual reactions: one had repeated withdrawal-like symptoms of general skin itching and eye symptoms approximately 3 weeks after injection, and the other had facial itching. The symptoms disappeared approximately 24 h after a following injection.

No SSE developed during reduction in *seasonal dosage*.

Apart from *increase in dosage* (before MTD) SSE depended on *high dosage* and *tissue sensitivity*. Major SSE developed in one patient at 500 BU, otherwise in 12 patients (92%) major SSE developed at high dosage (single dose) $\geq 1,000$ BU. Nasal thresholds below 1 HEP before treatment were detected in 62% (8/13) of the patients with major SSE during treatment but only in 22% (6/27) without major SSE ($P < 0.05$), but major SSE did not correlate to skin thresholds.

Whereas SSE were not significantly correlated to age, sex, individual symptom scores, allergen-specific IgE and IgG or anti-IgG (19, 20, 24), there was a tendency towards correlation with a high response in CRIE units (25).

Local side effects

Local skin reactions during *preseasonal therapy* before the first grass pollen have been presented previously (19). Only 18% (7/40) of the patients had local reactions of immediate type (within 30 min). Of these, only four patients had local reactions of 2–3 cm in diameter and three patients had local reactions of more than 3 cm. Delayed local side effects (DLS) detected in 70% (28/40) of the patients had début after 1–2 h, generally at maximum after 6–8 h, but most DLS disappeared within 24 h. If the dosage was repeated, the area of DLS was reduced approximately 50%. After injections, 91% (38/40) of the patients had local skin itching starting after 1/2–2 h, mean duration 48 h (range 1/2–72 h).

DLS had début in 43% (12/38) of the patients at ≤ 500 BU. Eight (40%) of the patients treated with the major allergens followed standard dosage right up to maximum (single dose)

10,000 BU, but two of the cases were complicated by urticaria and five by DLS. When treated with partially purified timothy extract, only five (25%) patients reached maximum dosage but one of these developed urticaria and two developed DLS. During further perennial treatment all patients showed MTD below 10,000 BU (21).

Local reactions and local side effects were of equal type *during subsequent perennial therapy*. Only 5% (2/40) of the patients had local reactions of immediate type, approximately 2 cm in diameter. Diameters of delayed local reactions during MTD just before each season are illustrated in Table 2.

An increase in delayed local reactions was observed after the first grass pollen season, and was significant ($P < 0.01$) in group PPA. Just prior to season 1, delayed local reactions predominated in group WPA ($P < 0.05$) but the number of patients with DLS in the two groups was equal. Later no significant difference in delayed local reactions or DLS was found between the groups, although the initially lower delayed local reactions in group PPA continued to be reflected. One patient showed consistently DLS in his right arm (right-handed).

DLS did not predict SSE, but tended rather to *negative correlation* since only 21% (6/28) of the patients with preceding DLS at lower dosage showed SSE compared with 50% (6/12) of the patients without DLS. Immediate local reactions did not correlate to delayed local reactions or SSE.

DLS before and during MTD did not significantly correlate to age, sex, individual symptom scores, serum-specific IgE or IgG, anti-IgG,

Table 3

Number of patients with subcutaneous nodules (SN) during maximal tolerated dosage (MTD) in group WPA and group PPA just before the three seasons. Significant differences between the groups were shown in 1979 and in 1980 at 5% level

	Group WPA	Group PPA
1978	1	1
1979	11	4
1980	12	3

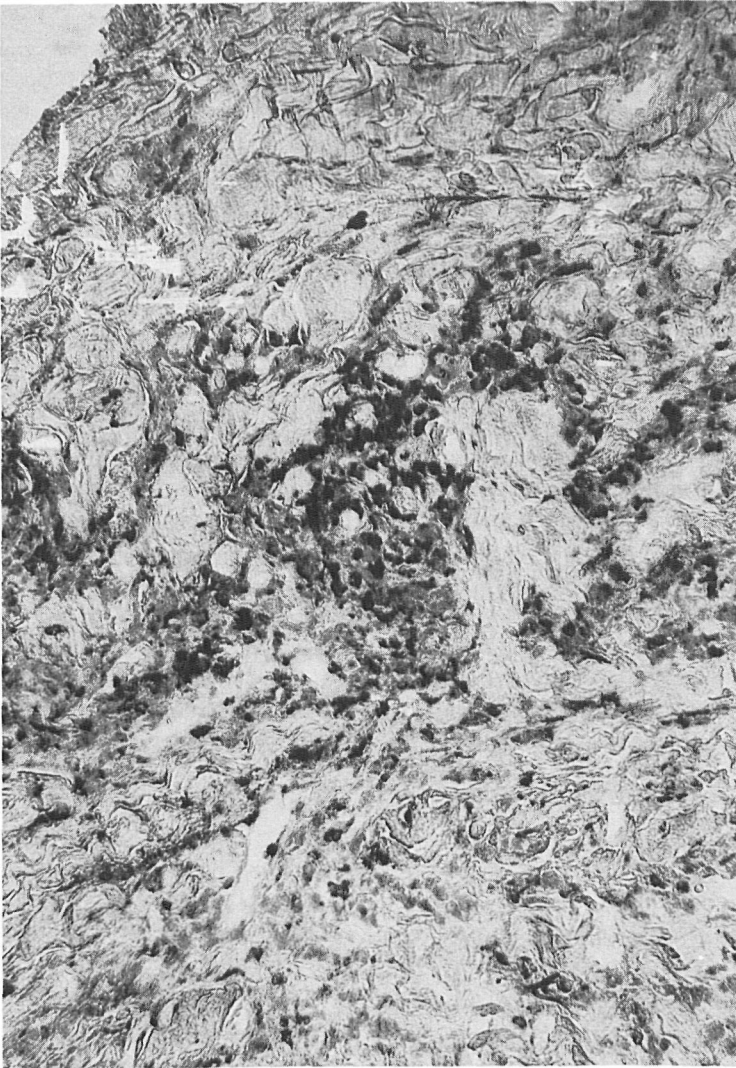


Fig. 1. Histological picture of a subcutaneous nodule (mag. $\times 150$). The deep layer of dermis shows a degeneration of the collagenous fibres mixed with epithelioid histiocytes forming a granuloma. The epithelioid histiocytes are stained (dark spots on this picture) using immunochemical technique (PAP) showing a clear content of muramidase (lysozyme).

high-response in CRIE, or pretreatment skin or nasal thresholds (19, 20, 21, 22, 23, 24, 25).

Subcutaneous nodules

Table 3 shows that only 5% (2/40) of the patients had subcutaneous nodules (SN) just before season 1, in both cases at a single dosage of 5,000 BU. During later MTD 38% (15/40) had SN. Prior to seasons 2 and 3 group WPA re-

cordd significantly more (four-fold) patients with subcutaneous nodules.

Generally, SN were maximally pea-sized and indolent, though in two patients they were approximately 1 cm in diameter. While most SN during MTD first disappeared within 2–3 months and because the injection sites were the same throughout the treatment, it was often difficult to distinguish between old and new nodules. Two patients had itching after in-

jection in the contralateral nodules, and one of these had slight urticaria above the nodules both at the injection site and contralaterally. A third patient had intermittent itching above the injection site for 1 week after the injection, and a fourth patient had itching in relation to heavy seasonal allergen exposition but with no relation to the injection. One patient had local hypoaesthesia for approximately 1 week, and two patients had superficial intracutaneous nodules, objectively bluish red and indolent but without skin necroses. No SN occasioned change of dosage.

A pale pink, well-defined subcutaneous nodule of 0.5 cm in diameter was excised (S.U.). Microscopy (S.L.) revealed in the deep layer of dermis a degeneration of the collagenous fibres to a bluish red colour, isolated in a scantily celled, finely granulated and structureless matrix, mixed with epithelioid histiocytes forming dispersing confluent granulomas where the cytoplasm with epithelioid histiocytes is mixed with a small number of plasma cells and lymphocytes. One of the granulomas is demonstrated in Fig. 1 where the epithelioid histiocytes are stained by means of an immunochemical technique (PAP) showing a clear content of muramidase (lysozyme). No necroses, no giant cells, no palisade arrangements, and no signs of malignancy were found. Immunofluorescence microscopy (S.U.) revealed no deposits of IgG, IgM, IgA, and C3-complement.

DISCUSSION

Systemic side effects

Latency in side effects with aluminium extracts raises general problems in comparison with aqueous extracts in accordance with the present study. Whereas side effects (LS and SSE) from aluminium bound extracts most often have their début after 1–2 h, with aqueous extracts they usually appear immediately in the clinic. In a recent publication on aluminium extracts general practitioners observed SSE in only 16% of the patients while 31% of the patients did so on retrospective evaluation (31). In so far as the patients during treatment with

aluminium extracts have SSE of the same character as the actual atopic complaint or have uncharacteristic minor SSE, it is difficult to determine whether it is in fact SSE that are involved. Thus some studies have shown a frequency of SSE in placebo-treated patients of 0% (9), 3% (7), 10% (12), and 19% (30), while the frequency in the placebo group has not been given in other studies (4, 10, 15, 18).

SSE seem to appear during *increase in dosage* levels. In this study major SSE developed in 33% of the patients before season 1 but only in 3% during subsequent perennial therapy. Similarly, Ransom et al. (28) reported SSE in 12.5% of the patients receiving aluminium extracts during the first year, but only in 4.5% during the second year. During the first 2 years of hyposensitization Nelson (17) found SSE in 0.1–0.4% and 0.6–0.9% of the injections with aluminium and aqueous extracts, respectively, but no SSE during MTD in the subsequent 2 years. In an investigation with biologically standardized aqueous timothy extracts Frostad et al. (8) used a more cautious dosage than in the present study, but in return no SSE were reported.

Of importance is whether the patients are given dosage up to MTD or merely to a low *dosage level*. This agrees with the present study where major systemic side effects appeared at high dosage (single dose) of $\geq 1,000$ biological units. Eisenberg et al. (5) showed clearly dosage-related SSE with pollen hyposensitization using dosages of $> 5,000$ PNU (20%), 2,500–5,000 PNU (10%), 1,250 PNU (6%), and $\leq 1,000$ PNU (0%). Taylor et al. (30) found 19% of patients with SSE after aluminium bound extracts of house dust plus mites, but also 19% in the placebo group. However, the patients were only given doses up to 250 PNU. In a large multicentre investigation by Vervloet et al. (34) with 19,739 patients, an average of 7.7 injections was given per patient, and the incidence of SSE was only 0.1% of the injections.

The *type and purification* of allergens may also play a role as shown by Vervloet et al. (34) where SSE were four to five times more frequent with pollen hyposensitization than with the other allergens. Eisenberg et al. (5) were not

able to show a difference in SSE incidence with various allergen types. In the present study, no certain differences in SSE incidence could be proved between treatment with PPA (2-antigen components) or WPA (20-antigen components from the same grass). Nevertheless, there does seem to be a preponderance of minor SSE with PPA (Table 2). On the other hand, Lichtenstein et al. (10) found only 10% SSE in patients treated with Ag E major allergen from ragweed versus 27% with crude extract of ragweed, and in another study (11) by the same authors four times more SSE with crude extracts (all extracts were aqueous). No certain preponderance of SSE was shown with crude extract as opposed to partially purified extract (7, 8).

The quality of the allergen extracts also plays a role in the development of SSE. The fact that the frequency of SSE in this investigation was relatively high is possibly attributable to the biologically standardized extracts used here because it has been ensured that the allergen extracts employed contain the active allergenic molecules.

SSE depends also on *tissue sensitivity*. In contrast to Eisenberg et al. (5) the present study did not show any correlation between skin thresholds and SSE, but most patients with major SSE were predicted by high nasal sensitivity (thresholds below 1 HEP before treatment).

No SSE in the present study were detected as the result of errors, but actual *errors* can certainly also be involved in hyposensitization. Vervloet et al. (34) found that errors were responsible for nearly 50% of SSE. Even though SSE with aluminium extracts are usually of the delayed type, it is important to observe the patients for a minimum of 30 min after injections as with aqueous extracts, since some attacks of anaphylactic shock have also been described with these extracts. To the author's knowledge the following seven occurrences of anaphylactic shock from aluminium extracts are described: two cases (3), one case (29), two cases (31), and two cases (34). Of these, one with début after 12 h (29), the rest within a few minutes. One of the cases was due to overdosage (34), another possibly to intra-

venous administration (31), but no errors were involved in the other cases. Including the two cases from ref. (31), the Danish National Health Service has registered four cases of anaphylactic shock from aluminium extracts, one of these in 1979 was fatal and concerned injection with aluminium extracts of house dust-fortified mite and cat dandruff (3).

Method of incidence evaluation is also decisive and can be misleading. In the present investigation 33% (13/40) of the patients had major SSE before MTD and 3% (1/39) of the patients major SSE during MTD, or SSE in 2.7% and 0.1% of the injections, respectively. There were 0.4 (16/40) major SSE per patient – 0.5 (20/40) if rhinitis was included (Table 1). Correspondingly, Rudolph et al. (29) found SSE to occur in 2.4% of the injections with aluminium extracts, and Normann et al. (18) found 1.1% with aluminium extracts – 3.3% with aqueous extracts. The latter authors have also given the number of SSE per patient, 0.19 with aluminium extracts and 0.58 with aqueous extracts. The number of patients with major SSE should be specified as otherwise many SSE relapses in a few patients will give a distorted picture.

Most researchers find fewer SSE with extracts coupled to *adjuvants*. Previously, oil emulsions were those most often used, but these have now been superseded because of suspected carcinogenesis in mice (26). The adjuvant in general use now is aluminium hydroxide which reduces the number of injections required for MTD, and, probably, the incidence of SSE compared with the incidence when using aqueous extracts. Thus Normann et al. (18) found 0.58 SSE per patient being hyposensitized with aqueous extracts versus 0.19 SSE with aluminium extracts. Others have found the following incidences of SSE (percentage of patients, with at least one SSE). Aqueous extracts: 30% (7), 30% (12), 46% (4), 32% (28), versus aluminium extracts: 22% (15), 0% (9), 19% (30), 2% (32), 13% (first year) (28), 5% (second year) (28), 7% (35), 31% (31), and the present study 50% (major SSE plus rhinitis before season 1), or 3% (major SSE plus rhinitis during subsequent perennial therapy).

Local side effects

Local side effects (LS) can not be said to be an unexpected reaction (a genuine side effect) since dosage levels are traditionally determined by LS.

Evaluation of the incidence of LS is encumbered with the same uncertainties as for SSE. In addition the definition of LS varies from clinic to clinic and differs with the use of aqueous or aluminium extracts. It is likely that aluminium extracts give fewer SSE, but more frequent and larger LS (DLS) than aqueous extracts (18). However, since LS are of immediate type with aqueous extracts and delayed type with aluminium extracts this comparison may not be relevant. Nor can it be excluded that immunological reactions may vary in kind.

It would seem understandable if the 20-component extract (WPA) during MTD before season 1 (Table 2) were to give greater delayed local reactions than treatment with the 2-component extract (PPA), but after season 1 this intergroup difference of local reactions tapered off. In fact, for both extracts (WPA and PPA), Ag 19 and Ag 25 were the most essential allergens for the majority of patients, and the initially intergroup difference can primarily be explained by the fact that most minor SSE were in the PPA group. This led to a more cautious (slower dosage increase) dose determination just prior to the first MTD registration (19).

In spite of a decrease in serum-specific IgE to pretreatment level or below (22) there was an increase in delayed local reactions after season 1 (Table 2) leading to reduced dosage in both groups (21). Provided delayed local reactions were conditioned by specific IgE, there would have to be a dissociation between cell-bound and free IgE during hyposensitization, and it has been shown by Malling et al. (14) that IgE cell-binding, in spite of hyposensitization, only increased during seasonal pollen exposition.

Since specific IgG blocking antibodies, in contrast to IgE, continued to increase during MTD (22) and since most local reactions were of delayed type, these reactions could be in-

fluenced by specific IgG even though IgG could not be proved in the biopsy of a single subcutaneous nodule.

The group with major SSE without DLS is an especially potentially risk-filled one, but could usually be preselected with low pretreatment nasal thresholds. Lack of DLS in connection with SSE could not be explained on the basis of injection errors since, firstly, the staff administered injections to all patients before MTD and since, secondly, a lack of DLS in these patients was a general phenomenon during the entire dosing phase both before and during MTD. The patients measured DLS themselves after 6–8 h, including the maximum reaction within a 24-hour period. But it is not probable that only patients with major SSE systematically measured too low local reactions. General lack of DLS was not attributed to low dosage since the majority of these patients followed the standard dosage levels before MTD up to 1,000–2,000 BU just as the other patients without SSE, and since dosage levels moreover were somewhat higher than in patients with SSE + DLS.

Subcutaneous nodules

Baumgarten et al. (2) have described in detail SN in relation to hyposensitization with aluminium extracts and reported the same frequency of nodules as in the present study. They have proved aluminium deposits in the vacuoles of the macrophages, and concluded that granulomas are caused both by immunological reactions and by foreign body reactions due to SN stimulation by seasonal pollen exposition in some patients (this was the case in only one patient in the present investigation).

The finding of aluminium deposits in the granulomas is supported by the current investigation with two extracts containing aluminium hydroxide at a concentration of 1:4 in equipotent biological doses and with the development of a proportionate frequency of nodules (Table 3). The abscesses and skin necroses characteristic in guinea pig trials (33) have not been shown. The granulomas described in biopsy were histologically in the order of 4–6 weeks old (2).

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