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CUTANEOUS REACTIONS TO POLLEN
EXTRACTS IN PATIENTS WITH HAY FEVER.
SPECIAL REFERENCES TO GRASS POLLEN
RECOMMENDED FOR TREATMENT IN
FIXED COMBINATION

By

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In the Swedish flora there are more than 100 species of grass (14). In a series of investigations made in the 1940's, Juhlin-Dannfelt (17, 18, 19) showed e.g. the types of grasses which, in particular, caused clinical symptoms of hay fever. A study of the conditions especially in Northern Sweden was presented by Heijer & Göransson in 1968 (13). In their study it was pointed out that there are reasons to look upon rye grass (*Lolium perenne*) as an allergen for Southern Sweden.

Our study is meant to clarify this point and at the same time to study the sensitizing properties of some other grasses. Some of these grasses are present in a standard combination for the hyposensitization of hay fever (Allpyral®, 5-Grass Mix). It has been demonstrated by Marsh et al. (15, 16, 23) that there are chemical as well as immunological similarities and differences between rye grass and some other grasses. The major allergen in rye grass pollen cross-reacts with the corresponding major allergen in most allergenic grass pollen, except for timothy and Bermuda grass. It appears to us that the next question would be whether the difference between rye grass and timothy appears as an absent or only slight, overlapping reaction when using standard extracts, i.e. if the

specific allergen(s) dominate in cutaneous extracts for routine use or if other (common) allergens assert themselves.

MATERIAL AND METHODS

133 patients (100 men and 33 women) with seasonal allergic rhinitis were followed. The age distribution was 9–53 years, and the average age 24 years. The patients had had hay fever in June–July for at least 2 years and had not been hyposensitized previously.

Intracutaneous skin testing was performed as serial dilution testing (titration) with aqueous extracts. The original extracts—concentration 1:1,000 w/v—were obtained from AB Vitrum, Sweden. For the titration the following grass pollen extracts were used:

Cocksfoot or Orchard / *Dactylis glomerata*
 June Grass or Kentucky Blue / *Poa pratensis*
 Meadow Fescue / *Festuca pratensis*
 Meadow Foxtail / *Alopecurus pratensis*
 Rye Grass or Perennial Rye / *Lolium perenne*
 Timothy / *Phleum pratensis*
 Wild Chervil / *Anthriscus silvestria*
 Yorkshire Fog or Velvet / *Holcus lanatus*

0.1 ml of the respective extract was injected intracutaneously on the outer aspect of the arm, and the criterion for a positive reaction was an urticarial change, after 15 min, of the size 10×10 mm or larger (2).

Primarily an extract in the concentration of 1:100,000 (10^{-5}) was injected. When the reaction was positive we continued with more diluted extracts in multiples of ten (1:1,000,000 = 10^{-6} , 1:10,000,000 = 10^{-7} , and so on up to 1:100,000,000,000 = 10^{-11}), until a negative reaction was obtained. When the reaction was negative to the concentration of 1:100,000 (10^{-5}), we continued with more concentrated extracts, 1:10,000 (10^{-4}) and 1:1,000 (10^{-3}). Thus the dilution end points were determined (12a), i.e. the weakest dilution which gave a positive skin reaction. The result of the skin test was recorded giving the exponent of the dilution end point (12b). In all cases, 0.1 ml of histamine (1:10,000) and Coca's solution were injected.

In patients with dilution end points of 5 or less, nasal provocation tests were performed. Pollen grains were insufflated in small quantities into the nasal cavities, and the reaction was judged after 15 min with regard to secretion, oedema and sneezing. The skin test and each provocation test were carried out on different days during a pollen-free period in 1972, and the patients did not receive any anti-allergic treatment at the time of the investigations.

RESULTS

The outcome of skin testing patients with hay fever due to grass pollen is given in Table 1. One hundred and thirty-three patients were examined, though only 80 patients took part in the test with rye grass. All pollens gave positive skin tests in more than 75 per cent of the patients, and seven of the grasses, viz. cocksfoot, June grass, meadow fescue, meadow foxtail, rye grass, timothy and Yorkshire fog, in 89 per cent or more of the patients. Rye grass and timothy had the highest reaction rate.

TABLE 1
Cutaneous Reactions to Grass Pollen Extracts in 133 Patients.

Grass	Positive* intra-cutaneous test per cent	Mean of exponents in serial dilution test
Cocksfoot	92	5.2
June grass	90	4.4
Meadow fescue	95	5.7
Meadow foxtail	92	5.6
Rye grass**	98	6.6
Timothy	96	6.4
Wild chervil	76	3.5
Yorkshire fog	89	5.5

* Positive = + reaction for 1:1,000 or more diluted solutions.

** Reactions to rye grass pollen were examined in 80 patients.

Table 1 also shows the average of the index for the dilution end point of each pollen. The same seven pollens, except June grass, reach the highest average figures. In the remaining six grasses (cocksfoot, meadow fescue, meadow foxtail, rye grass, timothy and Yorkshire fog) the index in titration was higher than 5, i.e. a positive intracutaneous test in higher dilution than 10^{-5} . Rye grass and timothy had the highest average values with 6.6 and 6.4, respectively.

The material with the index 5 or less which was positive in nasal provocation testing or had the index 6 or higher in titra-

tion with cocksfoot, meadow fescue, meadow foxtail, rye grass, timothy and Yorkshire fog are shown in Table 2. For cocksfoot the material consisted of 114 patients; for meadow fescue 122; for meadow foxtail 115; for rye grass 70; for timothy 118; and for Yorkshire fog 116. The table shows that there is a strong overlapping for the rye grass and timothy pollen extracts. Of the rye grass allergic patients, 97 per cent had a positive skin reaction to very diluted timothy extracts.

TABLE 2

Intracutaneous Reactivity to Rye Grass Pollen and Timothy Pollen in Patients Allergic to Various Grass Pollens.

Allergic to:	Number of patients	% of entire material**	Positive* skin reaction to	
			Rye grass %	Timothy %
Cocksfoot	114	86	87	92
Meadow fescue	122	92	99	97
Meadow foxtail	115	86	87	93
Rye grass	70	88	100	97
Timothy	118	89	97	100
Yorkshire fog	116	87	86	89

* Reactions to (alternative 1) solutions 10^{-6} or weaker or to (alt. 2) solutions 10^{-6} or stronger. In alternative 2, allergy was confirmed with provocation test.

** Entire material 133 patients, apart from rye grass with 80 patients examined.

The same applies to patients allergic to timothy pollen when their sensitivity to rye grass pollen is tested. Further, it can be seen from the table that patients allergic to cocksfoot, meadow fescue, meadow foxtail and Yorkshire fog have a similar skin reaction to rye grass pollen extracts and timothy pollen extracts, inasmuch as more than 85 per cent of these patients react to both.

DISCUSSION

Freeman (10) showed as early as 1911 that there is a cross-reactivity between different grass pollens, and he drew the

conclusion that timothy pollen extracts used alone were sufficient for skin testing and hyposensitization of grass pollen allergies. The same opinion was presented in 1943 by Juhlin-Dannfelt (17), and in 1955 by Frankland (9).

Chemical, immunological and also clinical investigations in 1940-1960, however, support treatment with multiple allergens. In 1948, Wodehouse (25) found that pollen antigens have a mosaic-like structure and consist of one major allergen which is species or group specific and has a number of minor antigens which are common to related and unrelated species in an unpredictable way. The minor antigens are capable of producing clinical symptoms. In 1953, Goesselin et al. (11) postulated the serological identity for extracts from 6 common grass pollens (cocksfoot, false oats, meadow foxtail, rye grass, timothy and Yorkshire fog). But in all probability the individual pollen extracts vary in their content of the common antigens which they possess, and at the same time possess certain qualitatively distinct antigens characteristic of their own individualities. In 1959, Rosa Augustin (4) showed that most—but not all—of the allergenic components in timothy pollen were also found in cocksfoot, and that there was a certain variation concerning the presence of determinants when compared with 9 other grasses. Feinberg (8) claimed, after his investigation in 1960, that out of 11 common grass pollens (including 7 of the grasses in the present investigation), each pollen contains one or more antigens unique to the respective pollen and which do not cross-react with antibodies against other grass pollens. These unique antigens cannot in any way be considered as minor allergens. In a series of investigations by Marsh et al. (15, 16, 23) of the chemical composition and structure and of the allergenic determinants in rye grass, it was shown that similarities and differences exist between rye grass and other grasses both chemically and immunologically. The major allergen in rye grass pollen cross-reacts with the corresponding major allergen in most other allergenic grass pollens, except for timothy and Bermuda grass. When registering the symptoms in nasal provocation tests, Mandall (22)

found, in 1967, that not all patients who were sensitive to a particular allergen showed the same reactions. Each pollen induced reactions that varied in localization and intensity from patient to patient. It was unusual for two pollens to provoke the same combination of symptoms in an individual, even though the pollens were morphologically similar and botanically related. For each individual patient, provocation tests with several allergens had to be performed for the provocation picture to correlate with the clinical picture of allergic rhinitis. Heijer & Göransson (13) improved the results of hyposensitization from 54 per cent to 94 per cent of the patients when they switched from single- (timothy) to multiple-pollen therapy. This verifies the hypothesis that treatment of hay fever with multiple extracts is preferable.

In today's range of Allpyral extracts there are standard mixtures of grass pollen. The uses of these are related to the need for several grasses. Allpyral 5-Grass Mix, in particular, is very commonly used (26). It contains cocksfoot, meadow fescue, rye grass, timothy and Yorkshire fog. One of the questions for our investigation was, whether the choice of these species of grasses was representative for our requirements in Southern Sweden.

Previous investigations support such an assumption to a certain extent. Juhlin-Dannfelt (19) found that cocksfoot, false oats, meadow foxtail, timothy and wavy hairgrass show the highest frequency of high skin titers. This, in addition to the results from pollen counts (which have shown that cocksfoot, false oat grass, hairy oat grass, meadow foxtail, rye (*Secale cereale*) and timothy have an abundant production of pollen), makes it probable that these are of special importance for hay fever. Heijer & Göransson (13) showed that intradermal tests in 100 hay fever patients in Northern Sweden were 100 per cent positive to meadow foxtail, 98 per cent to cocksfoot, 97 per cent to Yorkshire fog, 96 per cent to meadow fescue, and 95 per cent to timothy. According to Brown (6), most English hay fever patients are sensitive to the commonest grasses (cocksfoot, meadow fescue, rye grass,

timothy and Yorkshire fog) and much less to others. Our investigation supports the claim that the grasses included in Allpyral 5-Grass Mix to a large extent cover the conditions in Southern Sweden (Table 1). All 5 grasses in Allpyral 5-Grass Mix gave positive reactions in serial dilution testing in 89 per cent or more of grass pollen allergic patients. The average index for the dilution end points was 5.2 or more. The number of patients with a manifest allergy to one or more of these grasses was 86 per cent or more of the material. Our results also indicate, however, that many grass pollen allergic patients in Southern Sweden are also allergic to meadow foxtail, which is a common grass in this area (21) with an abundant pollen production (19). It could possibly be of value to add this grass to make a 6-Grass Mix.

In pollen-induced (timothy, birch, chrysanthemum) allergies, Juhlin-Dannfelt (20) found a high correlation between provocation tests and skin tests, which suggests a high reliability of skin reactions in pollen allergies. Arner et al. (3) and Brown (6) later confirmed this. With a titration technique, Colldahl (7) showed that the higher the skin sensitivity, the larger the percentage of positive results from the provocation tests. The titration technique thus permits an evaluation of the degree of sensitivity with the practically important conclusion: the higher the skin titer, the more probable a manifest allergy—investigations with modern immunological techniques, RAST, show analogous conditions, i.e. the higher the RAST titer, the more probable is a manifest allergy (1, 5, 24, 27).

Regarding our question, whether the demonstrated immunochemical dissimilarities between rye grass and timothy (23) are reflected in clinical work through a weakly pronounced overlapping in skin testing, the skin titration technique was used in the selection of the material. However, in some of the patients we carried out provocation tests in order to confirm the allergy. Thus the material consisted of patients with hay fever, where either the skin test was positive to dilution 10^{-6} or less, or positive to dilution 10^{-5} or more with a positive nasal provocation test. The results in intracutaneous skin test-

ing with rye grass pollen and timothy pollen are shown in Table 2. There was a good correlation when investigating the two pollen extracts. The immunological difference shown by Marsh et al. was not demonstrated in our skin tests with commercial extracts, but common and related allergens seem to dominate.

SUMMARY

Skin reactivity to 8 common grass pollens was investigated in patients with hay fever using commercial, unfractionated extracts in serial dilution technique. The highest degree of sensitivity was observed for cocksfoot, meadow fescue, meadow foxtail, rye grass, timothy and Yorkshire fog. Five of those are included in the Allpyral 5-Grass Mix.

The cutaneous responses to rye grass pollen overlap with those to timothy in a high degree.

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