

The effect of viability or non-viability of grass pollens on the biological activity of extracts prepared from them

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Summary

Freshly dehiscid pollens from four common grasses were each divided into two portions. One portion of each was placed in an extracting fluid within 2 hr of being shed (viable pollen) and the other portion was kept at room temperature in a desiccator for 2 weeks (non-viable pollen) before extraction. The relative skin test activity of each pair of extracts was determined on grass pollen sensitive patients.

No significant difference was detected between the extracts of the viable and non-viable pollens in any of the four species examined.

Introduction

Pollens have many interesting properties; they are, for instance, highly specific in that the pollen of one plant is not capable of fertilizing the ova of any but the most closely related plants. For example, timothy grass pollen cannot fertilize cocksfoot grass ova, nor those of brome, etc., so that each pollen must have a specific composition peculiar to its kind.

In addition to this species specificity, pollen grains lose their ability to fertilize their own kind within a relatively short time of dehiscence.

The viability of a pollen grain is probably best assessed by its ability to grow a pollen tube under suitable conditions of temperature and humidity.

The fact that pollen grains lose their viability on storage clearly indicates that a change has taken place; this led us to speculate as to whether freshly dehiscid viable pollen grains might have a more powerful biological effect than stored, non-viable pollens on appropriately sensitized humans.

If, as the result of such a change, a difference in allergenic activity could be demonstrated between viable and non-viable pollens, the methods of preparation of pollen extracts for diagnosis and treatment of pollen allergies might well have to be revised.

In order to determine whether there is a difference in reactivity between the extracts prepared from fresh pollens and those from stored pollens it was decided to

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compare the skin test reactions of such extracts in human volunteers known to be sensitive to them.

Jolliffe (1967) has shown that the viability of pollen grains falls off rapidly after 24 hr of storage under normal summer conditions and that there was no viability after 72 hr storage under these conditions.

Viability is judged by the ability of the pollen grains to grow tubes when kept at 100% humidity at 35°C for 1 hr.

On the basis of this work, pollen collected and used within 2 hr of being shed was regarded as viable, and that kept at summer room temperature, in a desiccator for 2 weeks was regarded as being non-viable. It is perhaps interesting to note that Jolliffe found that the colorimetric methods described by Vieitez (1952) using 2:3:5-triphenyl-tetrazolium chloride and Marks (1954) using an improved acetocarmine glycerol jelly technique were completely valueless in differentiating viable from non-viable grass pollens in the cases that she examined, and that Shardakov's (1940) modification of the Nadi test did not consistently confirm the results of pollen tube observations.

Materials and methods

Four grass pollens were used in this investigation: brome (*Bromus mollis*), red top (*Agrostis gigantea*), timothy (*Phleum pratense*) and Yorkshire fog (*Holcus lanatus*).

Separate plots of land had been sown previously with the seed of pure strains of the above-mentioned grasses. Just as the plants were about to come into bloom, a sufficient quantity of each grass was cut to enable 20 g of the pollen of each to be collected within 2 hr of harvesting.

The stems of the cut grass were placed in troughs of water arranged so that the flowering heads would overhang clean sheets of paper upon which the pollen could fall.

In each case 10 g of pollen was weighed and placed immediately in 200 ml of extracting fluid comprising 50% v/v aqueous solution of glycerine and containing 0.5% of phenol as preservative. The second 10 g of each grass pollen was stored in separate desiccators for 2 weeks, after which each was extracted in precisely the same manner as the viable specimens.

The mixture of pollen and extracting fluid was shaken vigorously twice a day for 10 days after which the pollen grain residues were removed by filtration through a Watman No. 5 filter paper.

The clarified extract was finally sterilized by filtration under pressure through a 0.22 μ Millipore membrane filter. These extracts were termed 5% and were each diluted with an equal quantity of glycerinated extraction fluid to give a 2.5% extract (25,000 Noon units/ml).

The 2.5% extracts prepared from the four viable pollens and from the non-viable specimens of the same four pollens were used by the prick test technique to skin test fifteen volunteers known to be sensitive to grass pollens.

The method of testing and recording the sizes of the skin test reactions was as follows.

The tests were done on the inner aspects of the forearms of the volunteers. The prick test technique was used, and the test sites were spaced at least 1 inch (2.5 cm) apart, and labelled by the use of a ball point pen. One drop of the appropriate extract was placed on the arm and using a sharp pointed instrument a prick was made

through the liquid so as just to pierce the skin. A control test was done using the extracting fluid alone. The whole series of tests was repeated on the other arm of each volunteer. Five minutes after the tests had been made the excess test solution was removed from the test sites using a separate piece of cotton wool for each in order to avoid cross contamination. Exactly 15 min after the tests had been applied, the raised weals were carefully outlined, using a ball point pen. A piece of sellotape (Scotch tape) 1 inch (2.5 cm) wide was placed along each line of reactions so that its sticky side was against the skin, and in such a way that it covered the outlines of the reactions, and the identification marks. Each strip was gently rubbed to make good contact with the skin and then peeled off and placed, sticky side down, on a sheet of graph paper marked in millimetre squares. In this manner an impression of the ball point pen markings on the arm was transferred to the graph paper. This method, which was devised in our laboratories some years ago, provides a good permanent record that does not smear. The sellotape is left stuck to the graph paper, and it is an easy matter to count the number of millimetre squares within each outline, and thus to obtain a quantitative measure of the area of the reaction.

The areas of the corresponding tests on the left arm and right arm were added, and the sum divided by 2 to give the average weal size for the test material concerned.

The areas of erythema that surround the raised weals were neglected since they are frequently confluent, and, in any case, are not normally regarded as a measure of the size of the reaction.

Results

The observed weal areas associated with the viable and corresponding non-viable extracts were compared by means of paired 't' tests (Snedecor & Cochran, 1967). The paired 't' test was adopted because each patient was tested with both the viable and non-viable extracts.

No significant differences were detected at the 5% level of significance between the mean weal area associated with the viable extract and that associated with the corresponding non-viable extract. The results are summarized in Table 1.

Chemical analysis for protein nitrogen content of the extracts was undertaken and showed no difference between those of the fresh and the stored pollen in each case.

Table 1. Skin test reaction in mm² and statistical evaluation on fifteen patients

Pollen	Viability	Mean weal area (mm ²)	Standard deviation	Sample size	't' value
Brome (<i>Bromus mollis</i>)	Viable	46.3	45.0	15	1.772
	Non-viable	27.9	19.7		(<i>P</i> = 0.05)
Red top (<i>Agrostis gigantea</i>)	Viable	41.5	32.3	15	1.012
	Non-viable	48.1	27.7		(<i>P</i> = 0.05)
Timothy (<i>Phleum pratense</i>)	Viable	26.2	14.1	15	0.059
	Non-viable	25.9	26.2		(<i>P</i> = 0.05)
Yorkshire fog (<i>Holcus lanatus</i>)	Viable	45.0	26.0	15	0.421
	Non-viable	41.9	21.1		(<i>P</i> = 0.05)
Control for viable		1.8	3.6	15	1.391
Control for non-viable		0.6	1.6		(<i>P</i> = 0.05)

Double diffusion in gels using the Ouchterlony technique revealed no difference in precipitation pattern between extracts of viable and non-viable specimens of the same pollen when the antiserum to the corresponding whole pollen was used.

Conclusions

So far as the four pollens investigated are concerned, there seems to be no doubt that extracts from viable and non-viable specimens of the same pollens are statistically indistinguishable allergenically as judged by skin test reactions on sensitive subjects. Since the four pollens used were chosen at random from among the most common species giving rise to hay fever, it may not be too much of an assumption to suggest that the allergenicity of any pollen is not appreciably altered when it loses its viability.

It is realized that the conclusions arrived at are subject to one or more of a variety of considerations.

For instance, if the viability factor(s) is rapidly destroyed by nasal secretions the conclusions are probably correct.

If the antigenic determinants are unaltered when the pollen becomes non-viable, the conclusions are probably correct.

If, however, the viability factor(s) is destroyed by the extracting fluid, but not by the nasal secretions, there may, in fact, be a greater clinical activity in viable pollen grains compared with non-viable grains that we have not, as yet, found the means to detect.

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