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THE SIZE AND WEIGHT OF COMMON ALLERGENIC POLLENS

An Investigation of Their Number Per Microgram and Size Distribution

By

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An investigation to determine the number of grains of pollen per microgramme is not purely an academic exercise. This is because it is a matter of some clinical importance in relation to defining the optimum quantity of dry pollen which should be used for nasal provocation tests, as too small a dose will produce a false negative result, too large a false positive.

Previous experience in this Clinic (1) in defining species specific sensitivity to extracts of the twelve main grass pollens found in Britain had shown that the optimum provoking dose was 50 Noon units, equivalent to the extract from 50 microgrammes of pollen. The previously established figure for *Dactylis Glomerata* pollen (Cocksfoot) by Hyde in 1944 was 82 grains per microgramme, so that this dose of extract was equivalent to about 4,000 grains.

A sedentary person will have a pulmonary ventilation of about 6 litres of air per minute during 8 hours sleep, and perhaps an average of 12 litres per minute during the waking hours, giving a probable minimum figure of 14.4 cubic metres of air per day. At an unusually high pollen count of 500/m³ this sedentary individual would inhale about 7,200 grains, but many persons more active would inhale 10,000 grains or more per day at the peak of the season. At the beginning of the

season many grass pollen sensitive subjects will react mildly to a count of $20/\text{m}^3$, inhaling 300 or 400 grains per day.

The apparent discrepancy between the effects of a few grains of freshly shed pollen and the dose of extract required to provoke a sensitive nose in the winter suggested that stored pollen is relatively impotent, or extraction methods inefficient. Feinberg (5) had come to similar conclusions in 1933, postulating that natural exposure every day to a slowly rising pollen count might be more liable to produce symptoms than one massive exposure out of season.

It seemed logical, therefore, to attempt to determine this figure for the major grass and other pollens for the purpose of estimating the optimum provoking dose.

The Coulter Counter seemed the ideal instrument with which to obtain these figures as the haemocytometer method used by Hyde (3, 4) was too laborious and could not give an accurate size distribution, while this machine is capable of giving reproducible results as often as desired.

MATERIAL AND METHODS

Weighing

Small quantities of pollen were weighed out using an electronic microbalance accurate to $0.1 \mu\text{gm}$. Aluminium foil pans were used for weighing each sample, which with the foil, was placed in a 100 ml polythene beaker with lid. The relative humidity of the atmosphere was recorded at the time of weighing.

Humidity Effect

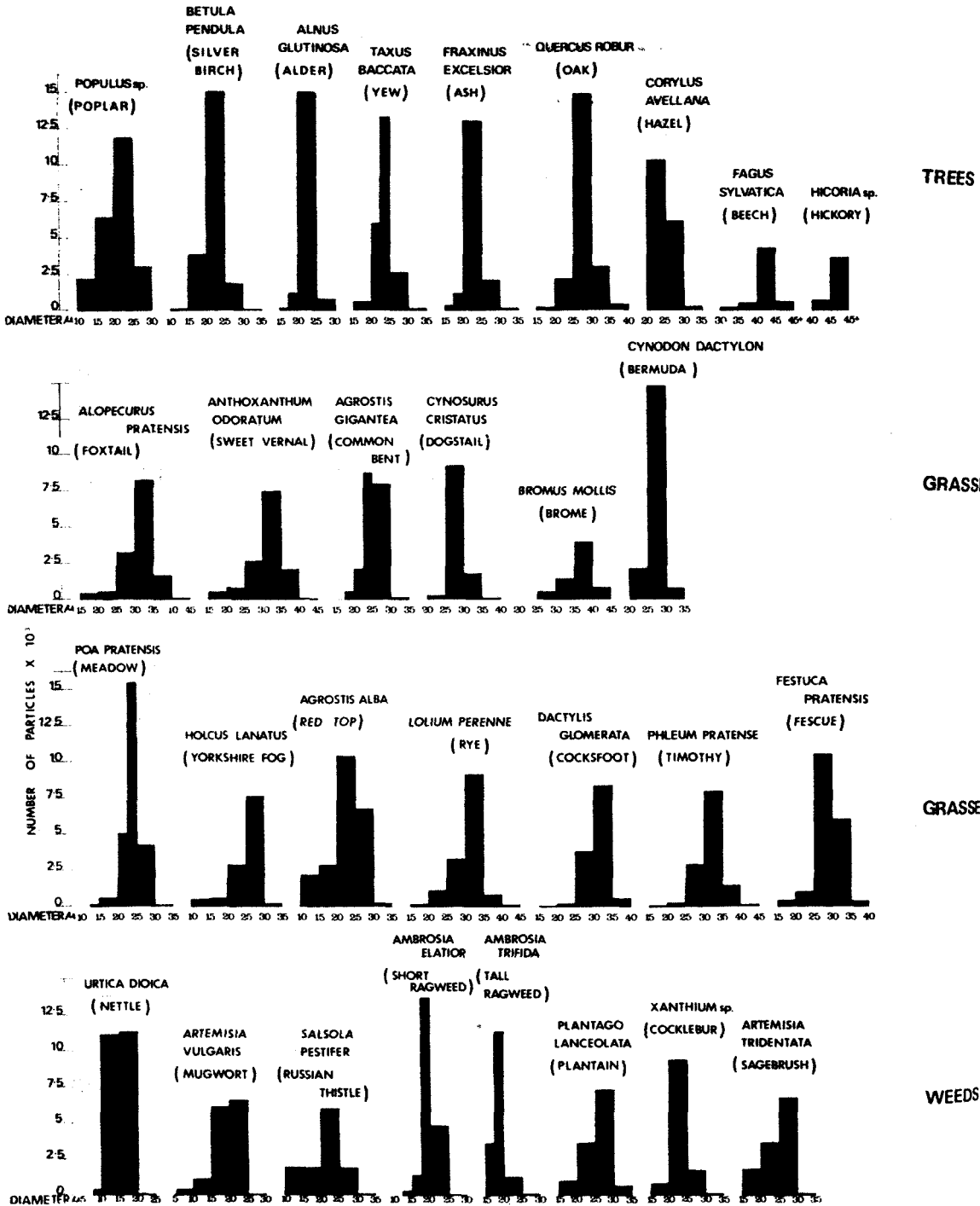
Plots of the weight of a *Dactylis* pollen sample against relative humidity showed a change in weight of 2 to 3 μgm per 1 per cent humidity per mg dry weight, the weight increasing with a rise in humidity and vice versa. All weights were converted to 55 per cent relative humidity using the figure 2.5 $\mu\text{gm}/\text{per cent change/mg}$ of sample. These hygroscopic changes were not noted by Durham (5) using very different methods in 1943.

The weights of pollen were adjusted so that in 100 ml of saline counts over 10,000 and under 40,000 were obtained, as between these values the counting error is only 1 per cent.

Preparing the Suspension

The bulk of the pollen was mixed with 4 drops of Tween 80 using a glass rod, and avoiding contact with the foil; 50 ml saline was then run into the beaker

POLLEN GRAIN SIZE DISTRIBUTION



over the foil, which was held by fine forceps. The pollen suspension was stirred and the final 50 ml saline run in over the glass rod. The lid was replaced and the suspension evenly dispersed by exposure to ultrasonic vibration for five minutes. Polyfusor 0.9 per cent saline (Boots Drug Co.) was used throughout as it has the lowest available background count.

TABLE 1
Number of Pollen Grains Per Microgramme.

Grasses

Dogs-tail (<i>Cynosurus cristatus</i>)	86
Rye (<i>Lolium perenne</i>)	68
Cocksfoot (<i>Dactylis glomerata</i>)	68
Timothy (<i>Phleum pratense</i>)	73
Bermuda (<i>Cynodon dactylon</i>)	113
Meadow (<i>Poa pratensis</i>)	175
Fescue (<i>Festuca pratensis</i>)	89
Foxtail (<i>Alopercurus pratensis</i>)	75
Common Bent (<i>Agrostis gigantea</i>)	169
Sweet Vernal (<i>Anthoxanthum odoratum</i>)	64
Brome (<i>Bromus mollis</i>)	35
Yorkshire Fog (<i>Holcus lanatus</i>)	120
Red Top (<i>Agrostis alba</i>)	202

Weeds

Nettle (<i>Urtica dioica</i>)	1003
Plantain (<i>Plantago lanceolata</i>)	124
Mugwort (<i>Artemisia</i>)	266
Giant Ragweed (<i>Ambrosia trifida</i>)	333
Short Ragweed (<i>Ambrosia elatior</i>)	293
Cocklebur (<i>Xanthium</i>)	187
Russian Thistle (<i>Salsola pestifer</i>)	142
Sagebrush (<i>Artemisia tridentata</i>)	132

Trees

Alder (<i>Alnus glutinosa</i>)	147
Hazel (<i>Corylus avellana</i>)	124
Ash (<i>Fraxinus excelsior</i>)	159
Yew (<i>Taxus baccata</i>)	238
Silver Birch (<i>Betula pendula</i>)	160
Beech (<i>Fagus sylvatica</i>)	23
Oak (<i>Quercus robur</i>)	93
Poplar (<i>Populus</i>)	145
Hickory (<i>Hicoria</i>)	19

Counting the Pollen

Using the Model A. Coulter Counter, the number of pollen grains in 0.5 ml suspension was counted, constantly observing the aperture throughout as partial blockage affects the count. It was impractical to have the solution stirred mechanically, but the suspension was stirred with a clean glass rod between successive counts.

Number of Counts Necessary

The makers suggest the average of two counts to be taken for figures over 10,000, but it seemed necessary to determine how many pairs of counts should be made on each sample to give a truly representative count.

Ten pairs of counts were made on *Dactylis Glomerata* (Cocksfoot) suspensions. This demonstrated that the result in grains per microgram varied by plus or minus one and sometimes two grains. It was, therefore, decided that six consistent counts per sample was more than adequate for accuracy.

Size Distribution

Prior to counting, the size range of the pollen was determined roughly by microscopic examination. The distribution peak was then determined on the counter, using specific size settings. The results are shown in Fig. 1.

Having established the remarkable accuracy of the method using *Dactylis* pollen, we proceeded to count other varieties of grass pollens, and extended the investigation to include the main pollens known to cause allergic symptoms.

Table 1 gives the numbers of grains per microgramme for all species tested. The close correlation of Hyde's (3) figures of 82 *Dactylis* and 975 for *Urtica*, when it is considered that he used the haemocytometer method, is remarkable.

S U M M A R Y

The number of grains per microgramme and the size distribution of the major allergenic pollens have been established. The results may provide a basis for the establishment of the optimum dose of these pollens which should be used as a provocation test before treatment, as a test of cure after treatment, and to decide if treatment is required before the coming season.

A C K N O W L E D G E M E N T S

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