

Table 1

Determination of individual, mean and median HEP values with a timothy pollen preparation in a population of 20 timothy-sensitive patients

| Patient    | Weal diameter (mm) |           | Individual HEP |
|------------|--------------------|-----------|----------------|
|            | Timothy            | Histamine |                |
| 1          | 5                  | 8         | 0.1            |
| 2          | 5                  | 6         | 0.4            |
| 3          | 5                  | 6         | 0.4            |
| 4          | 5                  | 6         | 0.4            |
| 5          | 5                  | 6         | 0.4            |
| 6          | 5                  | 5         | 1.0            |
| 7          | 6                  | 6         | 1.0            |
| 8          | 4                  | 4         | 1.0            |
| 9          | 6                  | 5         | 2.8            |
| 10         | 6                  | 5         | 2.8            |
| 11         | 8                  | 6         | 3.6            |
| 12         | 8                  | 6         | 3.6            |
| 13         | 8                  | 6         | 3.6            |
| 14         | 8                  | 5         | 10.0           |
| 15         | 8                  | 5         | 10.0           |
| 16         | 8                  | 5         | 10.0           |
| 17         | 10                 | 6         | 11.8           |
| 18         | 10                 | 5         | 32.8           |
| 19         | 10                 | 4         | 187.5          |
| 20         | 20                 | 5         | 912.2          |
| Mean HEP   |                    |           | 59.8           |
| Median HEP |                    |           | 3.2            |

allergen preparation. Aas & Belin (1) originally suggested a calculation procedure based on means rather than medians, but we find means poorly suited due to the skewness mentioned.

Even with median HEPs, though less than with mean HEPs, the result will be affected by the choice of population. If, for instance, the five most sensitive patients had been omitted from the population group of Table 1, the median HEP would have been reduced from 3.2 to 1.0. The mean would have fallen from 59.8 to 2.7. As might be expected, we obtained higher median HEPs when an allergen was assayed using hospital out-patients than using teenagers selected only for the presence of a weal with a diameter of at least 3 mm. The difference was roughly 4-fold.

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## Standardization of the RAST Inhibition Assay

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The radioallergosorbent test (RAST) is basically a technique for the determination of specific IgE antibodies (2). The allergens are coupled to an activated solid support. Serum from the atopic patient is added and the antibodies are bound to the solid phase allergens. The bound IgE is detected by <sup>125</sup>I labelled anti-IgE. The basic method is easily modified to be used for the assay of allergens or for the determination of

allergen extract potency. A RAST based direct titration and a RAST inhibition assay (3) have been used in research and routine analysis in our laboratories for several years.

The IgE antibody, i.e. the serum from the allergic patient, is the critical reagent of both methods. There is a considerable variation in the specificity of the antibodies of individual sera. This polymorphism can be



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## IgE-Based Radioimmunoassays for the Quantitation of Allergens

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For many allergens there is now good evidence that the corresponding allergy is IgE antibody mediated. Since the IgE allergen interaction can be quantitated conveniently by radioimmunoassay such as the radioallergosorbent test (RAST) these procedures can in principle be used for allergen quantitation. I will briefly review advantages and disadvantages of such procedures, of which the principles are given in Table 1. Emphasis will be mainly on two problems specific for

allergen estimation by radioimmunoassay:

1. The IgE antibodies used are in general not monospecific.
2. Allergens with only one determinant ("monovalent allergens") cannot induce mast cell triggering, and might even inhibit such a process.

It is therefore of relevance to discriminate between monovalent and polyvalent allergens.

Table 1  
IgE-based solid phase procedures for allergen RIA

|                                  | Step 1<br>unknown allergen plus | Step 2              | Step 3   |
|----------------------------------|---------------------------------|---------------------|----------|
| <i>Inhibition tests</i>          |                                 |                     |          |
| 1 labelled reagent allergen      | S-aE-E+A <sub>R</sub> *°        |                     |          |
| 2 insolubilized reagent allergen | S-A <sub>R</sub> +E             | S-A <sub>R</sub> -E | +aE-°    |
| <i>Binding tests</i>             |                                 |                     |          |
| 3 labelled unknown allergen      | °                               | A-°                 | +S-aE-E  |
| 4 insolubilized unknown allergen | S (reactive)                    | S-A                 | +E +aE-° |
| 5 insolubilized anti-allergen    | S-aA                            | S-aA-A              | +E +aE-° |
| 6 insolubilized anti-IgG         | S-aG +aA+E                      | S-aG-aA-A-E         | +aE-°    |

S, solid phase. aE, anti-IgE. E, IgE anti-allergen. A<sub>R</sub>, reagent allergen. A, unknown allergen. \*<sup>125</sup>I. aA, IgG anti-allergen. aG, anti-IgG.

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