

## Some Characteristics of Allergenic Components in an Aqueous Extract from Hazel Pollen (*C. avellana*)

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An aqueous extract from hazel pollen was enriched with respect to allergenic components by ultra-filtration followed by gel filtration on Ultrogel AcA54<sup>®</sup>. Crossed immunoelectrophoresis (CIE) performed with this solution revealed 15 precipitates. Twenty-eight identical CIE-plates were incubated with diluted sera from 28 different patients suffering from hazel pollen allergy, according to the traditional procedure for crossed radioimmuno-electrophoresis (CRIE) as described by Weeke & Löwenstein (3). The CRIE-reference system described by Aukrust & Aas (2) was used to classify the radiostaining intensities of the precipitates (1).

Fig. 1 shows the radiostaining distribution of the 15 precipitates. Control CRIE-plates with sera from patients not sensitive to hazel pollen were all weaker than CRIE-class G. The figure shows the occurrence of two major allergens associated with precipitates Nos. 10/11 and 6. These were named allergen HI and allergen HII, respectively. The antigens in precipitates Nos. 2 and 14 must be regarded as minor allergens. There is significant correlation between the radiostaining intensities of precipitate Nos. 10/11 and 6. Precipitate No. 10/11 always gave the most intensive radiostaining, while precipitate No. 6 ranged one or two CRIE-classes lower. It should be noted that these two precipitates did not show immunological identity reaction, judged by two different rabbit antisera. Furthermore, the radiostaining could not be due to co-precipitation in any of the precipitates, in view of the fact that they were positioned next to each other in the CIE/CRIE picture.

Preparative isoelectric focusing made it possible to separate allergen HI into two molecular forms. These variants, being both immunologically and allergologically identical, only differed with respect to their isoelectric points, which were 5.40 and 5.75, respectively. Similarly, heterogeneity was shown with allergen HII, giving two variants at pI 4.85 and 5.0. The pI 5.40 form of allergen HI was named allergen HIIa, and the pI 5.75 species, allergen HIIb.

Allergen HIIa, being quantitatively predominant, was isolated and shown to contain less than 5% of contaminants by several biochemical methods. This allergen species is allergologically active in skin prick test.

In RAST-inhibition experiments, the purified allergen HIIa totally inhibited the binding of IgE from a patient-serum-pool to RAST-discs containing crude extracts

from hazel pollen (to be published). This indicates that all the allergenic activity in a crude extract measured by RAST is represented in the purified allergen HIIa.

The correlation shown between radiostaining intensities of allergens HI and HII, and the inhibition capacity of allergen HIIa, led us to suggest that allergens HI and HII might carry the same binding sites for IgE. In order to clarify this possibility further, the technique of CRIE-inhibition was used. In principle, this method is identical to RAST-inhibition using CIE-plates instead of RAST-discs.

CIE was performed using the same extract that gave rise to the 15 precipitates mentioned. A rabbit antiserum, monospecific for allergen HI, was placed in an intermediate gel. This results in a better separation of the two distinctly different precipitates from allergens HI and HII. Prior to incubation with the CIE-plates, the serum-pool was incubated with a dilution series of

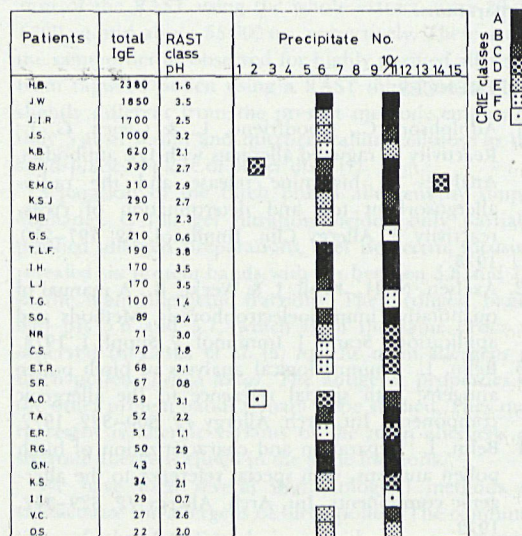


Fig. 1. Total IgE, specific IgE (RAST-class) and CRIE-radiostaining, corresponding to immunoprecipitates of hazel pollen antigens, due to the individual patient sera is shown. The intensity of the radiostaining is shown as dotted squares symbolizing the CRIE-classes from A to G.

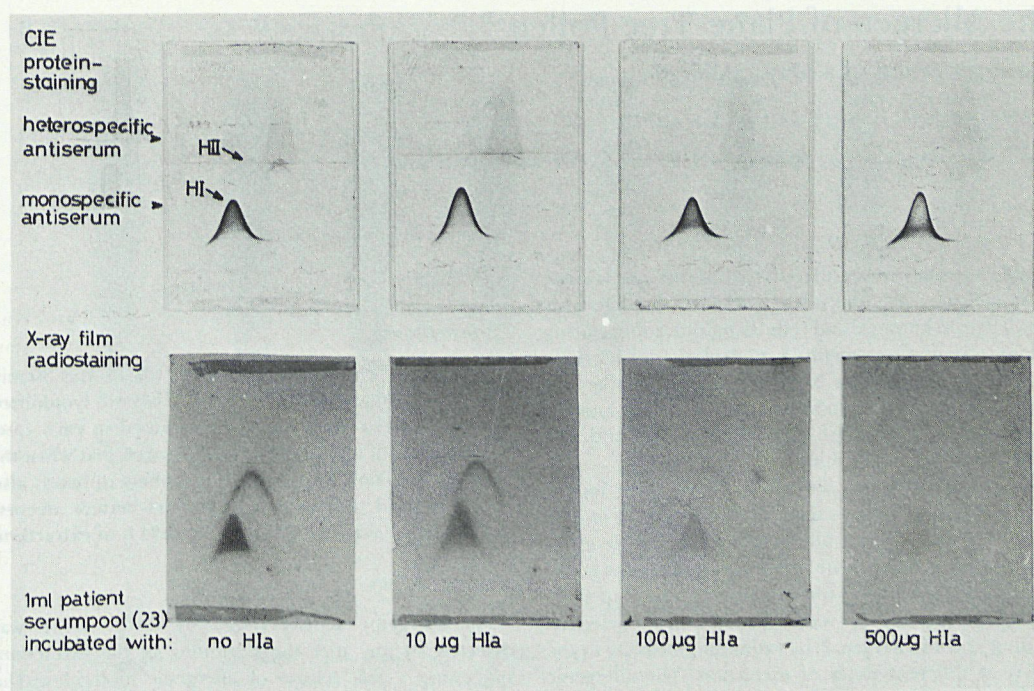


Fig. 2. Four identical CIEWIG-runnings of a hazel pollen extract containing both allergens HI and HII are shown. Antiserum monospecific for allergen HI was placed in an intermediate gel and a heterospecific antiserum in the upper gel.

The inhibitory effect of allergen HIIa on IgE-binding to precipitates of allergen HI and allergen HII are shown by the drop in radiostaining intensity obtained as the patient serum pool is incubated with increasing concentrations of allergen HIIa prior to incubation with the plates.

the purified allergen HIIa, thus blocking the IgE specific for HIIa. Fig. 2 shows how allergen HIIa inhibits the binding of IgE to the precipitate containing allergen HI, as would be expected. In addition, IgE-binding to the precipitate containing allergen HII was inhibited. This experiment implies that allergen HIIa includes all measurable IgE-binding sites of allergen HII.

In conclusion, rabbit antisera recognize allergen HI and allergen HII as two distinctly different antigens. But to IgE in a serum pool from 23 different patients, allergen HIIa is representative of allergen HII. This observation thus may suggest that IgE is less specific than IgG for the allergens in question.

#### REFERENCES

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