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ON THE OUCHTERLONY GEL DIFFUSION TECHNIQUE IN DEMONSTRATING THE OCCUR- RENCE OF ANTIBODIES IN ALLERGIC DISORDERS¹

By

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It is generally agreed that the presence of skin-sensitizing antibodies (reagins) in the serum can only be demonstrated by passive transfer by the method of Prausnitz-Küstner.

Other types of antibody have been demonstrated by methods like agglutination of collodion particles (1), agglutination of sensitized, tannin-treated erythrocytes (5, 6, 8), and gel diffusion (14).

The theoretical basis of these reactions is presumed to be that following binding to small particles, the antigen gives visible precipitation *in vitro* upon contact with the homologous, specific antibody.

So far, however, these methods have not yielded satisfactory results.

The gel diffusion technique, which in Ouchterlony's modification has gained ground in analysing complex antigens, has recently been employed in studying the occurrence of antibodies in bronchial asthma and hay fever.

Since these studies (*Rindom Schiøtt* 1953, 1954 and *Augustin* 1953) appear to show that sera from allergic patients give precipitates with ordinary allergen extracts, we

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adopted this method in our department in the hope that it might afford a valuable supplement to current allergic diagnosis.

In the study to be reported below we used sera from patients suffering from atopic dermatitis, bronchial asthma, and hay fever as well as sera from normal, non-allergic subjects.

MATERIAL AND METHOD

The gel was prepared as described by *Björklund* (1952), pH 6.9 and NaCl content 0.9 %. Using tetanus toxin—tetanus immune serum we obtained, within 24 hours, beautiful precipitation lines looking like vertical bands.

The allergen extracts (obtained from the Allergy Laboratory, Frihavns Apoteket, Copenhagen) consisted of 1 %, 5 %, and 10 % extracts of horse dander, dog hair, cat hair, cow hair, house dust, duck feathers, grass pollen, and hormodendron. All were freshly prepared, clear, and biologically active.

In the first experimental series a total of 31 sera were examined:

10 sera from patients with negative scratch tests,

15 sera from patients with many positive scratch tests, especially to house dust, grass pollen, feathers, horse dander, and hair from domestic animals,

6 sera from normal, non-allergic individuals.

During the experiments, the basins in the gel were filled on 3 consecutive days and the results read on the 10th day. Sterile instruments were used, as it was soon realized that the growth of bacteria might give rise to disturbing shadows in the gel.

Result: Allergen extracts of 1 % gave no precipitation. In the subsequently planned experimental series, carried out with 5 % and 10 % extracts, the first serum—derived from an untreated patient with hay fever—gave distinct precipitation lines with grass pollen extract. Considering the complete

absence of precipitates with the 1 % extract, we suspected that we might be dealing with non-specific precipitates and therefore started examining sera from normal, non-allergic persons. Table 1 shows the results of this control series.

It will be seen from the table that precipitates of varying intensity were observed with ordinary allergen extracts in 9 out of 10 normal subjects. The precipitates ranged in appearance from faint whitish stripes to distinct bands which were double in two instances.

SUMMARY AND CONCLUSION

Using the gel diffusion technique the authors investigated sera from normal, non-allergic subjects and from patients with atopic dermatitis, asthma, and hay fever, a total of 41 sera. Extracts were obtained of the most common inhalants (horse dander, house dust, hair from domestic animals, grass pollen, feathers, and hormodendron).

No precipitation occurred with 1 % extracts, whereas 5 % and 10 % extracts gave precipitation lines of varying intensity in 9 out of 10 non-allergic subjects.

These precipitates are presumably non-specific, as antibodies against the ordinary air-borne inhalant allergens are not likely to be so commonly present in the blood of normal persons.

The gel diffusion technique is hardly applicable in allergy for the purpose of distinguishing between allergic and non-allergic persons.

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