Abstract: A precipitation reaction by gel diffusion using sulfathiazole and serum of patients with suspected allergy to sulfonamides is described and the results and consequences discussed. It is concluded that this rather simple test may be helpful in cases of suspected immunization with drugs and that even small amounts of drugs absorbed from the intestinal tract may result in an allergic reaction provided the patient has been sensitized previously.

Skin reactions have been found in about 2% of patients treated with sulfathiazole (4). A skin reaction to sulfonamide is usually easily recognized, though it may be difficult, especially if a patient develops a skin reaction during simultaneous treatment with other drugs. In such cases it may even be impossible to identify the causative drug.

In this report a rather simple technique is described for the demonstration of antibodies to sulfonamides. It may be helpful in certain situations, provided the limitations mentioned in the discussion are borne in mind.

The inspiration for the present study was a 50-year-old woman without known allergies. She suffered from a generalized cutaneous pustulosis, which developed together with acute renal failure after 6 days of treatment with Italsept® (fthalylsulfathiazole). Italsept acts by a slow liberation of sulfathiazole in the intestinal tract. Absorption of small amounts of sulfathiazole may take place while Italsept per se is not absorbed to any significant extent (2). The patient was suspected to be in a state of allergic reaction, and since Italsept was the only drug administered before the onset of the disease, it was decided to test her serum for antibodies to sulfonamide. The presence of such immunoglobulins would indicate an immunization. Later on, 3 other patients were studied in a similar way.

MATERIAL AND METHODS

Survey of patients
2. A. K. P. aged 64. Suspected allergy to penicillin but not to sulfonamides. After abdominal operation for ovarian cyst, treatment with sulfamethoxazole + trimethoprim, 2 + 2 tablets a day. Subsequent increasing fever, rash and generalized pustulosis.
3. H. P. O. aged 84. Suspected allergy to penicillin and sulfonamides. Treated for urinary infection with sulfamethoxazole + trimethoprim for 5 days (dose not recorded). Two days after discontinuation, pustulosis of hands developed.
4. O. O. aged 77. Previous allergic reactions not recorded. Due to septicaemia (E. coli), treated with penicillin and streptomycin followed by sulfamethizole for 2 days and sulfamethoxazole + trimethoprim for 8 days (dose not recorded). After the discontinuation of sulfonamides the patient developed a generalized rash and pustulosis.

TECHNIQUE

Stock solutions of sulfathiazole in water containing 1 and 5 g per 100 ml were prepared. Two-fold dilutions with saline 0.9% were made and used as antigen in agar gel diffusion by Wadsworth's microtechnique (3, 5). A matrix of plexiglass with 6 basins (for antigen) placed around a central basin (for serum) was mounted with 2 pieces of insulation tape underneath at each side. A gel diffusion chamber (height 0.4 mm) was formed by placing the matrix on a 50 x 50 mm glass slide thoroughly cleaned and coated with agarose 0.3% in water. The chamber is shown in Fig. 1. One percent agarose was prepared using the following buffer: Barbital sodium g 7, NaCl g 6, 1 N HCl ml 22.7, distilled water up to g 1 000, pH 7.4.

A sufficient amount of agarose buffer at about 60°C was allowed to flow into the diffusion chamber from one of the open sides. After gelification, 30 μl of the reactants was pipetted into the basins according to the principle mentioned above. Diffusion took place for 48 hours in a moist atmos
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Precipitates were obtained with the highest concentrations of a 1% solution of sulfathiazole, as shown in Fig. 2. The close proximity of the precipitation lines to the wells containing antigen suggested a too-low concentration of antigen. By means of a 5% solution of sulfathiazole, the precipitates moved closer to the central well containing the serum (Fig. 3). No precipitation was seen using a serum from a normal person. The sulfanilic acid gave only precipitation with the serum from case 1. A survey is given in Table 1.

**RESULTS**

The presence of precipitating antibodies to sulfathiazole shows that the patients had been immunized by this drug. In case 1 the serum sample tested was drawn on the 9th day after the start of treatment with fthalylsulfathiazole. It must be concluded that the immune apparatus of this patient had been triggered by a sulfa-drug some time in her past, while the antibodies demonstrated during the present treatment were considered to be produced as a response to a secondary stimulus by small amounts of sulfathiazole absorbed from the intestinal tract. Logically, a serum sample from the time before the allergic reaction was not taken for control.

The presence of precipitating antibodies to a drug indicates that an immunization has taken place, but it does not prove the presence of an allergic state of a patient. On the other hand, the

![Fig. 1. Gel diffusion chamber (x 2).](image)

![Fig. 2. Scheme showing precipitation obtained with serum from case 1 (central basin) using serial dilutions of 1% sulfathiazole sodium (peripheral basins). A distinct precipitate is seen close to the basin containing 0.5% sulfathiazole sodium, suggesting a too-low concentration of antigen.](image)

![Fig. 3. Scheme showing precipitation of the serum from Fig. 2, but using serial dilutions of 5% sulfathiazole sodium. The precipitate is now located around the central basin and disappears at a concentration of 0.6%.](image)
Table I. Survey of results

<table>
<thead>
<tr>
<th>Patient</th>
<th>Skin reactions</th>
<th>Drugs</th>
<th>Presence of antibody to sulfathiazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. E. P. ♂</td>
<td>Generalized pustulosis</td>
<td>Sulfathiazole</td>
<td>+</td>
</tr>
<tr>
<td>2. A. K. P. ♂</td>
<td>Generalized pustulosis</td>
<td>Sulfamethoxazole +</td>
<td>+</td>
</tr>
<tr>
<td>3. H. P. O. ♂</td>
<td>Pustulosis of the hands</td>
<td>Sulfamethoxazole +</td>
<td>+</td>
</tr>
<tr>
<td>4. O. O. ♂</td>
<td>Drug (?) rash</td>
<td>Sulfamethazole +</td>
<td>+</td>
</tr>
</tbody>
</table>

The test may serve as indirect evidence, provided an allergic reaction to some drug is suspected. A prerequisite for the performance of the test is that the drug is soluble and stable in a suitable buffer at pH 7–8 at room temperature for at least 48 hours.

On the other hand, it must be emphasized that a negative result does not rule out an allergic reaction to the drug in question. This is related to the fact that the amount and affinity of precipitating antibodies may be too low for the establishment of a precipitation reaction to take place.

REFERENCES

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