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PRECIPITATING ANTIBODIES TO SULFATHIAZOLE IN SERUM OF PATIENTS WITH SUSPECTED ALLERGY TO SULFONAMIDES

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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 ftalysept® (fthalylsulfathiazole). Ftalysept acts by a slow liberation of sulfathiazole in the intestinal tract. Absorption of small amounts of sulfathiazole may take place while ftalysept per se is not absorbed to any significant extent (2). The patient was suspected to be in a state of allergic reaction, and since ftalysept 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

1. E. P. ♀ aged 50. Never allergic reactions. No earlier treatment with sulfonamides. After diagnosis of adenocarcinoma recti, preoperative treatment with ftalylsulfathiazole 1 500 mg × 4. On the 7th day generalized pustulosis, fever and renal failure.

2. A. K. P. ♀ aged 64. Suspected allergy to penicillin but not to sulfonamides. After abdominal operation for ovarial 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 × 50 mm glass slide thoroughly cleansed 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 gellification, 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-

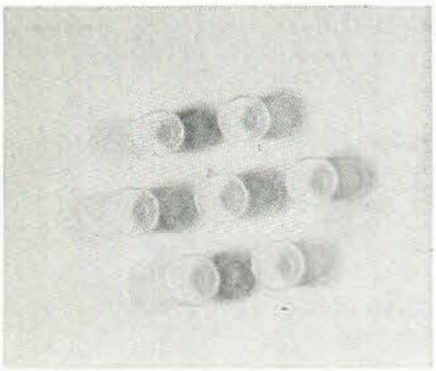

Fig. 1. Gel diffusion chamber (× 2).

phere with one refilling of all basins after 24 hours' diffusion and after preliminary recording of the results.

Obviously, one cannot expect the appearance of heavy precipitates in a study like the present one, as the short immunization will give a low antibody titre in the serum. Therefore, the results are most easily recorded by examination of unstained plates using a light source placed below and lateral to the plate, which is placed over a dark background. The precipitation lines appear white and the results may be recorded by drawing a picture of the precipitates. For storage and reproduction, staining is necessary. In this study Coomassie Brilliant Blue (1) was used. Before staining, the unprecipitated material was eluted by placing the plates in saline for 24 hours followed by another 24 hours in distilled water to remove salts. The plates were then covered with filter paper and dried in an oven at 60°C. The staining solution had the following composition: Coomassie Brilliant Blue, 5 g; ethanol 90%, 450 ml; glacial acetic acid, 100 ml; distilled water, 450 ml. For destaining, the same solvents were used.

Since the precipitates are rather weak the destaining procedure is critical, as too little destaining gives too little contrast and too much causes the colour of the precipitates to disappear. Therefore, the results must be recorded from the fresh, untreated plate after diffusion, and can only be considered positive if a linear, well-defined precipitate appears between the central basin containing the serum and one or more of the peripheral basins containing antigen. The experiments were repeated using the sodium salt of sulfanilic acid as antigen in a 5% solution. This compound was used to see whether the antibodies were directed against the central structure of the sulfonamide molecule.

RESULTS

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 I.

DISCUSSION

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

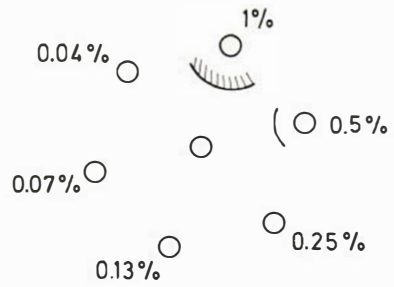

Fig. 2. Scheme showing precipitation obtained with serum from case 1 (central basin) using serial dilutions of 1% sulfathiazole sodium (peripheral basins). A distinct precipitate is seen close to the basin containing 0.5% sulfathiazole sodium, suggesting a too-low concentration of antigen.

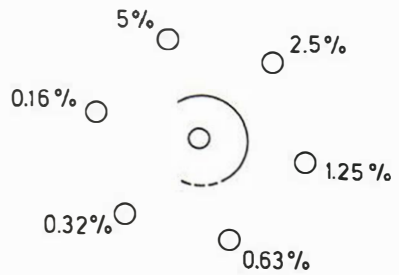

Fig. 3. Scheme showing precipitation of the serum from Fig. 2, but using serial dilutions of 5% sulfathiazole sodium. The precipitate is now located around the central basin and disappears at a concentration of 0.6%.

Table I. *Survey of results*

Patient	Skin reactions	Drugs	Presence of antibody to sulfathiazole
1. E. P. ♀	Generalized pustulosis	Ftalylsulfathiazole	+
2. A. K. P. ♀	Generalized pustulosis	Sulfamethoxazole + Trimethoprim	+
3. H. P. O. ♂	Pustulosis of the hands	Sulfamethoxazole + Trimethoprim	+
4. O. O. ♂	Drug (?) rash	Sulfamethizole Sulfamethoxazole + Trimethoprim	−

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.

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