

'FALSE POSITIVE' PRECIPITATION REACTIONS TO EXTRACTS OF ORGANIC DUSTS DUE TO A TEICHOIC ACID FROM *S. AUREUS*

TECHNIQUES

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(Received 28 May 1970)

SUMMARY

Precipitation reactions regarded as 'false positive' have been obtained to extracts of a variety of organic dusts. The antigen responsible for these reactions is an electrophoretically fast moving and negatively charged substance, previously observed in extracts of mouldy hay and other vegetable and organic dusts, and referred to as a 'D line' antigen. This antigen has now been shown to have antigenic determinants in common with a β -teichoic acid prepared from *Staphylococcus aureus*. Absorption of the sera with *N*-acetyl-D-glucosamine had an inhibitory effect on 'D line' and teichoic acid precipitin reactions.

INTRODUCTION

A wide variety of organic dusts of vegetable, animal, fungal and other origin stimulate the production of precipitating antibodies in man (Pepys, Longbottom & Jenkins, 1964). A correlation has been established between the relevant specific precipitins and clinical disease caused by the inhalation of some of these dusts, as for example in farmer's lung (Pepys *et al.*, 1963), bagassosis (Salvaggio *et al.*, 1966, 1969), and bird fancier's lung (Reed, Sosman & Barbee, 1965, Hargreave *et al.*, 1966). In addition to precipitin reactions, shown by inhalation tests to be specifically related to the particular disease, other, apparently unrelated, precipitin reactions have been found in tests with certain extracts against the sera of healthy and unexposed, as well as exposed, affected subjects. The commonest such reaction is given by an electrophoretically fast-moving, negatively-charged, antigen, first observed in this context in an extract of mouldy hay (Pepys & Jenkins, 1965), and since then, in certain extracts of the following: coffee (kiboko) dust; sugar-cane bagasse (Hearn & Holford-Strevens, 1968); certain cultures of *Dermatophagoides farinae* on dog-meal (Holford-

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Stevens *et al.*, 1970); mouldy barley; 'blackfat' (a semi-processed) tobacco; pigeon droppings, and cultures of *Glycyphagus domesticus* on dried yeast.

The specific antigens in mouldy hay relevant to farmer's lung and derived from *Microsporypha faeni* give precipitation reactions on immunoelectrophoresis in regions on both sides of the antigen well, termed A, B and C, whereas the faster-moving, 'non-specific', reaction occurs in the region termed D, this reaction being referred to here as the 'D line' and the antigen as the 'D line' antigen.

The presence of this 'D line' antigen in a contaminated sample of pigeon serum and in a pigeon droppings extract in routine use, and the subsequent isolation of *Staphylococcus aureus* from them, was the starting point for the identification of the 'D line' antigen. It is important to recognize the nature of such reactions to extracts of organic dusts and thus avoid 'false positive' interpretations.

MATERIALS AND METHODS

(1) Samples of mouldy barley, coffee (kiboko) dust, 'blackfat' tobacco, pigeon-droppings and a culture of *D. farinae* were defatted overnight in ether and were then extracted in Coca's solution. These extracts were Seitz-filtered, dialyzed overnight and freeze-dried (Pepys *et al.*, 1964).

(2) The strain of *Staphylococcus aureus* isolated from the contaminated pigeon serum was cultured on nutrient agar at room temperature for 7 days. The agar was frozen and thawed repeatedly. The fluid obtained was centrifuged and the supernatant was Seitz-filtered, dialyzed and freeze-dried. This is referred to as the *S. aureus*, pigeon serum isolate.

(3) An extract of *Staphylococcus aureus* provided by Professor J. R. May, Institute of Diseases of the Chest, London, was prepared by ultrasonic disintegration of the organism in saline. The supernatant after centrifugation was dialysed against running tap water for 48 hr and freeze-dried.

(4) An extract of teichoic acid prepared from *S. aureus* strain H, was provided by Dr A. B. Archibald, Department of Organic Chemistry, Newcastle. This was a ribitol teichoic acid with β -*N*-acetylglucosaminyl substituents on each ribitol unit.

(5) A preparation of *N*-acetyl-D-glucosamine (puriss. Koch-Light) was used for absorption of the positive sera.

The extracts were tested at concentrations of 1, 10, 20 and 30 mg/ml by agar-gel double diffusion and immunoelectrophoresis, according to the procedures used previously with extracts of organic dusts (Pepys *et al.*, 1964).

RESULTS

In double-diffusion tests with the sera of patients suffering from a variety of lung diseases, precipitin reactions were obtained on double diffusion against: coffee (kiboko) dust extract in twenty-six out of thirty-nine (66%) sera; *S. aureus* extract twenty-one out of forty-one (51%) and the teichoic acid in ten out of thirteen (77%).

The double diffusion test, Fig. 1, shows precipitation reactions of identity to the extracts of *D. farinae*, coffee (kiboko) dust, *S. aureus*, teichoic acid, mouldy barley and 'blackfat' tobacco, when tested against a human serum. Reactions of identity were also given by the above extracts, the *S. aureus* pigeon serum isolate, and a pigeon droppings extract. The same

human serum, however, gave no reactions to fresh pigeon serum or to an extract of fresh pigeon droppings.

The teichoic acid was tested at concentrations ranging from 0.1 to 20 mg/ml. Fig. 2(a)

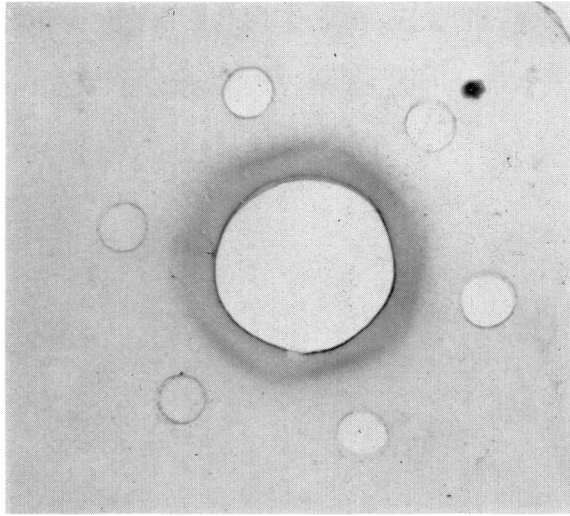


FIG. 1. Double diffusion test with a patient's serum showing the precipitin reaction of identity given to the following test extracts (clockwise from the black spot): *D. farinae* (20 mg/ml), coffee (kiboko) dust (10 mg/ml), *S. aureus* (10 mg/ml), teichoic acid (1 mg/ml), mouldy barley (30 mg/ml) and 'blackfat' tobacco (30 mg/ml).

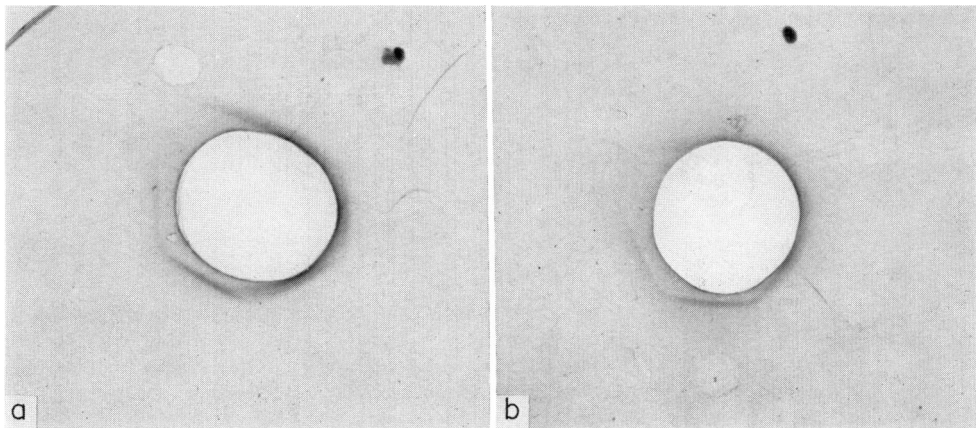


FIG. 2. Double diffusion test with the serum from patient (a) and patient (b) showing the precipitin reactions given with decreasing concentrations of the teichoic acid. (Clockwise from the black spot): Teichoic acid 20 mg/ml, 15 mg/ml, 10 mg/ml, 5 mg/ml, 1 mg/ml and 0.1 mg/ml. Note: that no precipitin reaction occurred with serum (b) at the higher concentrations of 20 mg/ml and 10 mg/ml.

shows a serum reacting to all but the lowest concentration, whereas Fig. 2(b) shows a serum which reacted to the 1 and 5 mg/ml concentrations, but not to the higher concentrations.

In immunoelectrophoresis tests, the contaminated pigeon serum, the extract of the *S. aureus* isolated from it, the teichoic acid, the *S. aureus* antigen, the extracts of coffee (kiboko) dust and 'blackfat' tobacco all gave precipitation reaction in the D region (Fig. 3).

The precipitin reactions to extracts containing the 'D line' antigen were shown to occur with the γ -globulin fraction of the serum, separated by the method of Levy & Sober (1960).

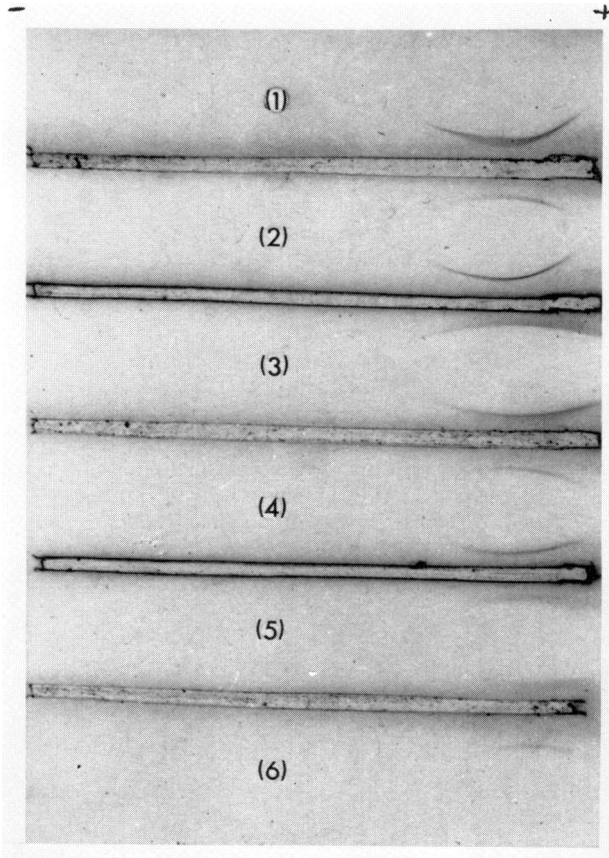


FIG. 3. Immunoelectrophoresis of the test extracts: (1), *S. aureus* isolated from the contaminated pigeon serum (10 mg/ml); (2), contaminated pigeon serum; (3), teichoic acid (10 mg/ml); (4), *S. aureus* (10 mg/ml); (5), coffee (kiboko) dust (10 mg/ml); (6), 'blackfat' tobacco (30 mg/ml) tested against a patient's serum showing a precipitin reaction in the fast moving 'D' region. The anode is on the right.

Absorption of a positive serum with either the teichoic acid or the coffee (kiboko) dust extract, resulted in the inhibition of the precipitation reactions of identity produced by the teichoic acid, the coffee (kiboko) dust extract, the extract of *S. aureus* and the pigeon droppings extract.

The effect of N-acetyl-D-glucosamine on the precipitation reactions, produced by the teichoic acid and the D antigens, was studied by adding 20 mg to 1 ml of the test serum. The reaction was inhibited completely in four and partially in one of the seven sera which gave

a precipitin reaction to the teichoic acid. Of 5 sera reacting to *S. aureus* and coffee (kiboko) dust extract, the N-acetyl-D-glucosamine absorption inhibited the *S. aureus* reaction in three and the reaction to the coffee (kiboko) dust extract in two. Of four sera giving reactions to the *D. farinae* and 'blackfat' tobacco extracts, the reactions were completely inhibited in one case.

DISCUSSION

The 'D line' antigen shown by Pepys & Jenkins (1965) in mouldy hay and since then detected in a wide variety of organic dust extracts, gave a reaction of identity with an extract of *S. aureus*, attributable to its content of teichoic acid.

Teichoic acids were first isolated from bacterial cell walls (Armstrong *et al.*, 1958). Those from *S. aureus* were shown to be polyribitol phosphate with α -, β - and α - β -N-acetyl glucosaminyl residues and D-alanine ester residues (Sanderson, Strominger & Natherson, 1962, Baddiley *et al.*, 1962, Haukenes, 1962a).

A teichoic acid with β -N-acetyl glucosaminyl residues prepared from *S. aureus* strain H contained antigenic determinants common to those of the 'D line' antigens. Absorption with the β -teichoic acid inhibited the 'D line' reaction in the sera tested and it would be of interest, in the light of the findings of Torii, Kabat & Bezer (1964), to test an α -teichoic acid in the same way. On immunoelectrophoresis, the teichoic acid and the other 'D line' antigens have been shown to be negatively charged and fast moving. This is in agreement with the findings of Haukenes (1962b) and Singleton, Ross & Kohn (1964) on the electrophoretic mobility of their teichoic acid preparations.

Reports on the incidence of precipitin reactions to teichoic acid vary. Singleton *et al.* (1964) showed that patients with burns had precipitins to teichoic acid and many workers have reported that only patients with staphylococcal infections have precipitins to *S. aureus* (Julianelle & Hartman, 1936; Rinker & White, 1964; Martin, Daugharty & White, 1965). Burns (1967) found precipitins to *S. aureus* in 15–22% of his patients with various chest diseases, and also in 9% of normal subjects. Martin *et al.* (1965) using the Preer tube technique showed that 79% of normals tested had precipitins to teichoic acid and Torii *et al.* (1964), using a very sensitive technique, demonstrated precipitins to teichoic acid in all the subjects tested. We found that between 50–70% of normal subjects have precipitins to the 'D line' antigens, the incidence depending on the extract and the concentrations used.

The concentration and purity of the antigen are important in double diffusion tests, as certain sera gives precipitation reactions to teichoic acid at a concentration of 5 mg and 1 mg/ml but not to higher concentrations of 20 mg/ml. Similar observations (Faux, 1968) have been made with another polysaccharide antigen, namely the cell wall mannan of *C. albicans* Group A. Only 28% of a group of sera reacted to this mannan when it was used at 10 mg/ml, whereas all the sera reacted to a 1 mg/ml concentration. Such concentration effects could explain the different values reported for the incidence of precipitins against *S. aureus* and teichoic acid.

The haptenic inhibition of the precipitation reactions of some of the sera to teichoic acid and to the 'D line' antigen in the various extracts, by absorption with N-acetyl-D-glucosamine shows, as reported by Torii *et al.* (1964), that it is one of the determinants in the teichoic acid. However, as reported by Torii *et al.* (1964), the specificity of the combining sites on the antibody may be directed against an N-acetyl glucosaminyl ribitol structure or larger determinants of the teichoic acid.

'D line' reactions to the different organic dust extracts used in the diagnosis of allergic

respiratory diseases caused by their inhalation, occur very frequently and must be distinguished from the specific reactions. Some evidence that the D antigen and the 'D line' are not relevant to the clinical disease is provided by Hearn & Holford-Strevens (1968). They found that inhalation tests with a D antigen preparation provoked no reaction in patients with bagassosis, who gave 'D line' precipitation reactions. The production of antibodies responsible for 'D line' reactions may be related to infection with *S. aureus*. The occurrence of the D antigen in the organic dusts is probably due to the presence of *S. aureus* or of substances with antigenic determinants related to teichoic acid.

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