PRECIPITATE ADSORPTION ON SURFACE (PAS): A NEW PRINCIPLE FOR SEROLOGICAL ANALYSIS

Hans ELWING
Institute of Medical Microbiology, Guldhedsgatan 10, S-413 46 Göteborg, Sweden

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1. Introduction

Precipitate adsorption on surface (PAS) is a method for identification of immune precipitate formed in a gel [1]. PAS is a combination of immunoprecipitation in gel and thin-layer immunoassay (TIA) [2,3]. The principle of PAS is that an immune precipitate formed in a gel, adsorbs on a supporting solid surface coated with antigen, that is related to the precipitate. Adsorption of the precipitate material may be visualized, after removal of the gel, by condensation of water on the surface. The adsorbed precipitate is observed as a hydrophilic condensation pattern at the former position for the precipitate in the gel.

Here, a simple method for investigation of relationship between antigens is described. The method is based on a combination of PAS and a technique of selective absorption of the precipitate forming antisera. As a model, antigenic relationship between human IgG and IgG from some other animals was investigated.

2. Materials and methods

2.1. *Antigen*

Serum from human, horse, rabbit, monkey, goat, guinea pig and chicken was used. To avoid possible interference with complement reacting with adsorbed IgG, the sera were investigated at 56°C for 30 min.

2.2. *Antiserum*

Swine-anti human IgG, heavy chain specific (batch Be-12) was a gift from Orion Diagnostica, Helsinki. Absorption of anti-IgG with animal sera in equal volumes was performed at 37°C for 1 h.

2.3. *Polystyrene petridishes*

Plates of 4.5 cm diam. were obtained from NUNC A/S, Roskilde.

2.4. *Method*

The plates were coated with serum diluted 1/200 in 0.15 M NaCl for 30 min at room temperature. They were then rinsed with distilled water and 1% agar dissolved in 0.15 M NaCl was poured onto the plates to form a layer 2.5 mm thick. Pairs of 3 mm basins 4 mm apart were punched in the agarlayer. One of the basins in each pair was filled with human serum diluted 1/20. The other was filled with anti-human IgG unabsorbed or absorbed with equal volumes of animal sera. After 20 h diffusion a single precipitate had developed between the basins. The precipitates were registered and the agar was rinsed off with distilled water. The plates were dried by means of blowing with compressed air. Visualization of adsorbed precipitates was performed by exposing the plates to water vapour for 3 s (vapour condensation surface (VCS) technique) [3]. Precipitate adsorption was registered as an increased wettability of the condensation pattern at the former position of the precipitate.

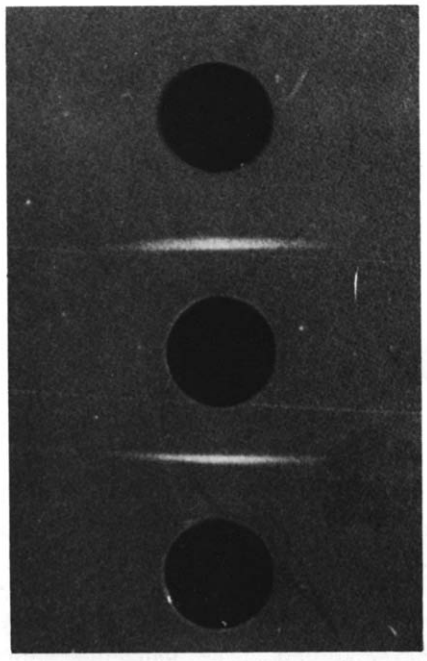
2.5. *Comparative double diffusion*

Comparative double diffusion in gel was performed with use of microplates according to [4]. Human serum diluted 1/50 and undiluted anti-IgG was used as reference. Animal sera was used undiluted.

3. Results

3.1. *The principle of the analytical PAS-method*

In fig.1 the result of an experiment with the PAS-



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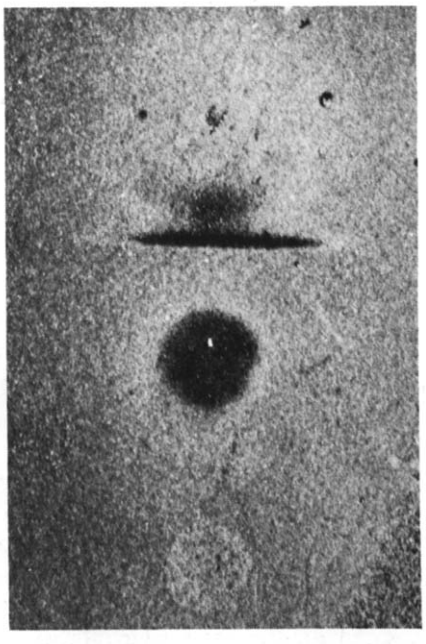

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Fig.1. The analytical PAS technique. (a) A photograph of the gel. (b) A photograph of the corresponding plastic surface with condensation pattern. For further explanation, see text.

technique is shown. The plastic surface was coated with horse serum. The middle basin was filled with human serum, the upper basin with unabsorbed anti-IgG and the lower basin with anti-IgG absorbed with horse serum. After 20 h of diffusion the precipitates were photographed (fig.1a). The agar was removed from the plates and adsorption of precipitates was visualized by condensation of water on the surface (fig.1b). It was observed in this experiment that IgG precipitate formed by unabsorbed anti-IgG adsorbed on the surface whereas precipitate formed by anti-IgG absorbed with horse serum failed to adsorb on the surface.

3.2. *Serological relationship between human IgG and serum components from other animals*

Plates were coated with serum from different animals as indicated in table 1. Pairs of basins were filled with human serum and anti-IgG unabsorbed or absorbed with animal serum as indicated in table 1. It was observed in this experiment that IgG-precipitates formed by unabsorbed anti-IgG was adsorbed on surfaces coated with serum from human, monkey, rabbit, goat and horse. This indicates a serological relationship between human IgG and components in these sera. IgG precipitates formed by anti-IgG absorbed with monkey serum were adsorbed on plates coated with human serum but not on plates coated with other serum. This indicates that a common antigen determinant of human IgG is shared by serum components from monkey, rabbit, horse and goat. IgG precipitates formed by anti-IgG absorbed with rabbit serum was adsorbed to all in table 1 indicated serum-coated plates except plates coated with rabbit serum. This may indicate the presence of an isolated rabbit determinant on human IgG. Such isolated goat and horse determinants were also found as indicated from the results of the experiment. Finally no adsorption of IgG precipitates was observed on plates coated with serum from guinea pig or chicken.

The antigentic pattern of human IgG as interpretated from the experiments is shown in fig.2.

3.3. *Double diffusion analysis*

A reaction of 'partial identity' of human IgG and monkey serum was found. Rabbit and horse serum formed weak and fuzzy precipitates with anti-IgG, which made interpretation of antigenic relationship with the reference IgG-precipitate difficult or impossible. Guinea pig, goat and chicken serum did not form visible precipitates with anti-IgG.

Table 1
Results obtained with the PAS technique in analysis of relationships between human IgG and serumcomponents from different animal sera

Anti-human IgG absorbed with serum as indicated	Plates coated with serum from:				
	Human	Monkey	Rabbit	Horse	Goat
Unadsorbed	+	+	+	+	+
Monkey	+	–	–	–	–
Rabbit	+	+	–	+	+
Horse	+	+	+	–	+
Goat	+	+	+	+	–

+ indicates adsorption of precipitates. For further explanation, see text

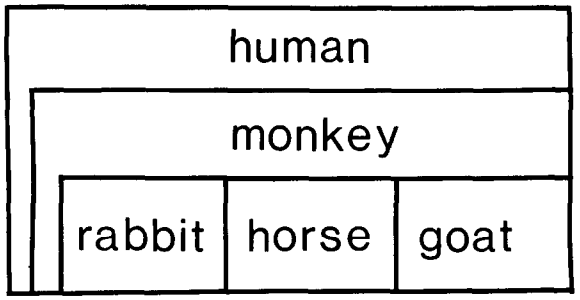

Fig.2. Antigen pattern of human IgG as interpreted from results obtained with the analytical PAS technique.

4. Discussion

The PAS-method and the precipitation in gel methods, e.g., comparative double diffusion in gel, may be regarded as 2 different principles for serological analysis. The main difference between the principles is that PAS implies a 2 phase system, solid–liquid (gel), whereas precipitation in gel methods implies a 1 phase system, the liquid (gel). It is known that the chemical and physical properties of the solid–liquid interphase differ from those of the solid as well from those of the liquid [5]. One such property of the interphase is the high increase of concentration of a substance at the interphase after adsorption of the substance to the solid. This common phenomena is the most probable explanation to the observed higher sensitivity and thereby greater analytical resolution of PAS compared to that of comparative double diffusion in gel.

The visualization method, water condensation, or vapour condensation on surface (VCS) technique used in this work is a very simple and sensitive method for registration of adsorbed precipitate material. The basic principles underlying the detection of protein layers on solid surfaces by the water condensation method is also fairly well understood [6].

It may however, be added that a prerequisite for accurate registration of adsorbed precipitate with the VCS-technique is that the adsorbed antigen molecules not are too closely packed on the solid surface.

If so, the binding of the diffusing antibodies and the antigen layer will be visualized as well which may cause difficulties in registration of precipitate adsorption. This phenomenon forms the basis for quantitation of antibodies with TIA and has been described in [1]. A method of avoiding the disturbing TIA reaction, is to mix the antigen preparation with antigenically non-related proteins which will compete with the antigen molecules during the adsorption and thereby prevent close packing of the antigen molecules on the solid surface. There was no need to perform this here probably because the serum, which was used for coating of the plates, contained protein molecules antigenically non-related to the IgG antigens.

Another, complication in PAS was that IgG precipitates of very high density frequently adsorbed on diverse serum coated surfaces irrespective of anti-IgG adsorption. This type of unspecific adsorption was easily avoided by dilution of the reference antigen and antiserum or by increasing the distance between the diffusion basins, methods which reduce the density of the precipitate. The complication described was by no means critical; it showed however, that an adsorption of a precipitate not could be judged as immunospecific in absence of relevant control experiments.

In studies of relationship between antigens, the described PAS technique has, in comparison with precipitation gel methods, both advantages and disadvantages. An advantage with the PAS technique is that only the reference antigen had to migrate and form precipitate in the gel, whereas the requirements of the test antigen is to be absorbed on the solid surface. The properties of being adsorbed on a solid surface is less restricted ability of an antigen compared to the ability to diffuse and form precipitates in the gel. This opens up a possibility also to perform PAS analysis of antigens which have poor precipitating or diffusing properties. A disadvantage with PAS is that the reaction of 'non-identity' observed in precipitation in gel methods has no counterpart in PAS.

The results obtained concerning antigenic relationships between human IgG and IgG (or other serum components) in different animal sera agree with those in [7]. With use of complement fixation method relations were found between human IgG and serum components from monkey and horse, but not with serum from chicken. However, the complement fixation method does not give a detailed picture of relationships between antigens, in contrast to PAS. The PAS principle may open up new research possibilities in serological analysis.

Acknowledgements

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