Detection of Antigen-Antibody Interactions by an Interferometric Method

THE most sensitive technique available for identifying proteins consists of using the protein, by injection into a suitable animal, as an antigen, and diffusing it through a gel containing the antibody produced by the animal. The antigen and antibody combine in certain proportions, and if they are present in sufficient amounts a band of precipitate is formed. Oudin¹ diffused one reactant into a gel in which the other reactant was evenly distributed. Ouchterlony² and Elek³ diffused both components towards one another through a gel. Complex mixtures of antigens and antibodies may be analysed by means of this technique since each specific antigen-antibody system behaves independently as though the others were not present. The principles of the technique outlined below have been described by Ambrose⁴ and the technique has been used by Conmar Robinson to examine the diffusion of solvents through polymers⁵.

The apparatus consists, essentially, of two glass plates, each optically flat on one surface, which is silvered to give about 5 per cent transmission of light. The two plates with their silvered surfaces facing one another are placed horizontally on a microscope stage, with a cell containing a channel filled with gel between them, and the plates adjusted until they are almost parallel. The plates are illuminated from below with parallel monochromatic light the angle of incidence of which may be varied by means of an adjustable pinhole. Interference fringes are observed, each fringe being a contour line of constant optical path, which with a uniform medium is the path of constant distance between the silvered surfaces. The silver is given permanent protection by a glass plate in the form of a narrow wedge, with its upper surface optically flat, which is cemented to the silvered surface. The inclined upper surface of the protecting plate makes the secondary fringes formed by reflexions between it and the silvered surface of the other plate so close together that they do not interfere with the appearance of the main fringes. With suitable plates, a set of straight parallel fringes can be obtained. If in any region there is, for example, an increase in refractive index, the straight fringes will bend towards the apex of the wedge in that region in order to keep the optical path constant, since an increase in refractive index must be compensated by a shorter distance between the plates. Therefore, if there is an accumulation of a complex between an antigen and an antibody in a particular region, and if this results in a change of refractive index, an inflexion in the fringes should occur in which the peak corresponds to the point of equivalence of the antigen and antibody. This has been observed. The quantity of antigen and antibody combined before the commencement of precipitation may be estimated from the area under the peaks and the thickness of the gel, since the values for the refractive indices and specific refractive increments are almost constant for most proteins⁶.

Photographs (a) and (b) show the fringe systems observed in a gel through which diphtheria antigen, AG, is diffusing from the left and antibody, AB, from the right. In photograph (a) a band of precipitate (B_1) has been formed on the right-hand side. In photograph (b), taken of the same system at a later stage, the band of precipitate (B_1) observed in photograph (a) has increased in width, and peaks in the fringes can be observed running across the channel

Fig. 1. Interference fringes showing specific precipitation of antigen-antibody complexes in gelatin

to the left of B_1 . These peaks (B_2) correspond to the point of equivalence of a second antigen-antibody system. No precipitate at B_2 could be detected when photograph (b) was taken. Before any precipitate had appeared in band B_1 it had the same appearance as band B_2 shown in photograph (b), giving peaks in the interference fringes, but being quite undetectable by other means.

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¹ Oudin, J., *C.R. Acad. Sci., Paris*, **222**, 115 (1946).

² Ouchterlony, O., *Acta path. microbiol. Scand.*, **25**, 186 (1948).

³ Elek, S. D., *Brit. Med. J.*, 493 (March 13, 1948).

⁴ Ambrose, E. J., *J. Sci. Instr.*, **25**, 134 (1948).

⁵ Robinson, Conmar, *Proc. Roy. Soc., A*, **204**, 339 (1950).

⁶ Adair, G. S., and Robinson, M. L., *Biochem. J.*, **24**, 993 (1930).