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DETERMINATION OF THE DIFFUSION CONSTANT OF POLIOVIRUS BY THE GEL PRECIPITIN TECHNIQUE

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SUMMARY

A gel diffusion technique is recommended for the determination of the diffusion constants of materials of which only small quantities are available. In the present work this technique has been applied to the investigation of the diffusion rate of a type II poliovirus strain. A diffusion constant of $1.29 \cdot 10^{-7}$ cm^2/sec at 20° was obtained for this virus. This figure is probably within $\pm 5\%$ of the true value, an assumption based on previous data obtained when the method was applied to the study of the diffusion constants of a variety of proteins.

INTRODUCTION

In a previous communication, VAN REGENMORTEL[1], reported on the determination of the diffusion constant of TYMV by the double gel diffusion technique of POLSON[2]. The value found by him agreed very well with the figure obtained by MARKHAM[3], who used the method of free diffusion in conjunction with the well known Lamm scale method.

Abbreviation: TYMV, turnip yellow Mosaic virus.

VAN REGENMORTEL conducted his experiments in 0.5 % agar gel in saline and as it appeared that this concentration of agar had no influence on the relative diffusion constant of virus and precipitating antibody, it was logical to assume that this concentration would also have no influence on the relative diffusion constants of poliovirus and its precipitating antibody. This assumption is based on the similarity in particle sizes of TYMV and poliovirus.

MATERIAL AND METHODS

The source of virus was a crude 1000 fold concentrate of infected monkey kidney tissue culture fluid of the Collans strain of Type 2 poliovirus which had been kept in the frozen state at —20° since 1955. Prior to storage the titre was $10^{9.8}$ $TCID_{150}$/0.5 ml. The virus was partially purified from 5 ml of this concentrate by three cycles of differential ultracentrifugation; 12,000 rev./min for 10 min to remove coarse tissue debris, 30,000 rev./min for 90 min to bring down the virus in a pellet and 8,000 rev./min for 10 min to remove coagulated matter from the redispersed pellet.

The immune serum used in the gel diffusion experiments was obtained from a monkey which had been hyperimmunized against the homologous antigen.

The diffusion experiments were conducted in a precision glass precipitin apparatus supplied by Messrs. L.K.B. Produkter (Stockholm), and in a similar apparatus made of perspex. Measurements on the positions and widths of the bands were made with a Hilger Watts microcomparator.

RESULTS

In Fig. 1a and b, are given photographs of the "perspex" gel diffusion apparatus after the diffusion process had proceeded for 8 and 60 days respectively. The white

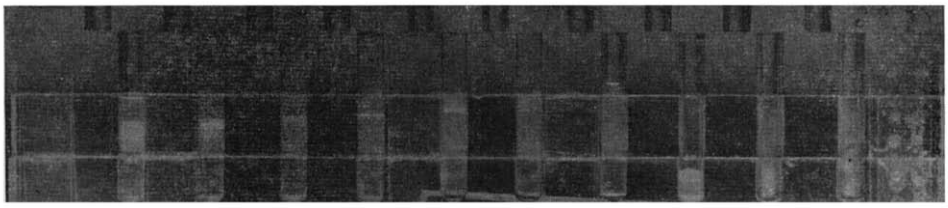

Fig. 1a. Photograph of gel precipitin apparatus eight days after start of experiment. Antibody at constant concentration placed in cups in bottom of apparatus in liquid state, neutral agar layer in central tubes and falling twofold dilutions of antigen in upper cavities. Note minimum band width in region of fourth column.

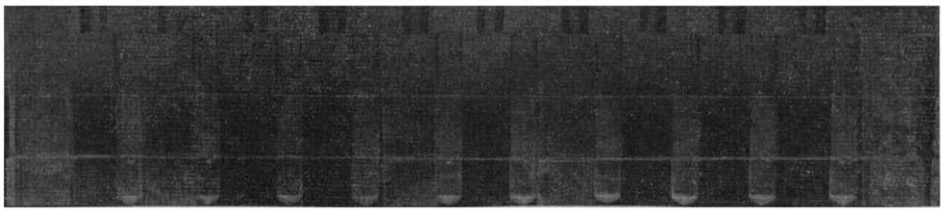

Fig. 1b. Photograph of gel precipitin apparatus 60 days after start of experiment. Note the broadening of bands. Band in fourth column still narrowest. Additional bands, probably artefacts, produced after too long standing.

lines across the tubes in the central section in (a) are the precipitin bands which formed in 0.5 % agar through the reaction of serial two-fold dilutions of the virus suspension in the upper cavities, with a 1 in 10 dilution of the precipitating antibody in the cavities in the lower part of the apparatus. The linear relationship between antigen concentration and distance of the precipitin band from the antigen menisci can be clearly seen. In (b) is shown to what extent the bands have broadened after 60 days of diffusion, and that in the region where optimal proportions exists between the reactants, the widening of the band was less than on either side of this region.

In Fig. 2 is given a set of results obtained with the "perspex" apparatus. The oblique lines across the diagram are the band distances from the antigen menisci after 4, 5, 12, 17 and 34 days of diffusion. This was done by plotting the distances

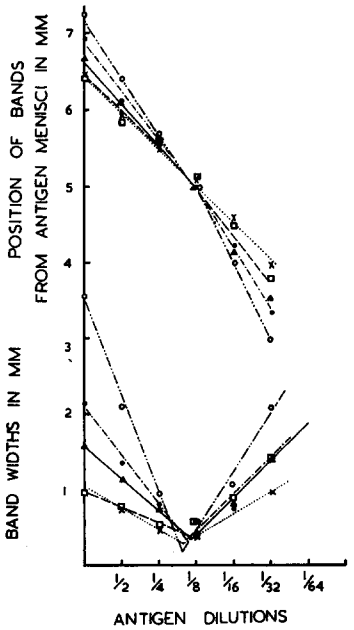

Fig. 2. Gel precipitin test on Type II poliovirus Collans strain. The dogainal curves show the average precipitin band positions, +, after 5; □, 6; △, 13; ●, 18, and ○, 35 days of diffusion plotted against the virus dilution. The V-shaped curves show corresponding variation of precipitin band widths plotted against virus dilution.

from the antigen menisci to the centres of the precipitin bands against the antigen dilutions. The V-shaped curves represent the widths of the corresponding precipitin bands as a function of the antigen dilution. It will be seen that the series of oblique lines intersect at a point 5.14 mm from the antigen meniscus. The position of this point corresponds very well with the average position where the limbs of the V-shaped curves intersect. On the left-hand side of the point of intersection of the oblique lines there is clearly a linear relationship between $\log_2$ antigen dilution and band position for all the lines. To the right of this point, however, the linear relationship does not hold for all the lines but shows a definite departure from linearity. This deviation tends to become progressively larger the longer the observation period was extended. The non-linear relationship may be explained by the fact that while the

advancing edges of the precipitin bands remain sharp, the trailing edges tend to become diffuse as a result of redissolving of the precipitate when the antibody is in excess. When measuring the lines the centres are sought at positions further removed from the antigen menisci with the consequent deviation from linearity. Judging from other systems examined this source of error is not apparent when the antigen is in excess, or when both reactants are present in larger amounts.

Diffusion constant

At the position of intersection of the oblique lines, or where the minimum band width is located by the V-shaped curves, the two reactants are present in exact optimal proportions in their respective containers.

TABLE I

DETERMINATION OF X_g FROM THE V-SHAPED CURVES*

Time of diffusion in days	*Interpolated position on 5 days line*
5	5.2 mm
6	5.12 mm
13	5.10 mm
18	5.125 mm
35	5.175 mm
Average	5.14

* See Fig. 2.

In Table I are given the positions of minimum band widths as interpolated on the oblique straight line obtained during the initial stages of the experiment.

The following equation was used for calculating the diffusion constant, POLSON[2], OUCHTERLONY[4].

$$D_g = \left(\frac{X_g}{X_b}\right)^2 \cdot D_b \qquad (1)$$

Where D_g and D_b are the diffusion constant of the virus and antibody, and X_g and X_b are the distances from the antigen and antibody menisci respectively where the precipitin band formed, when the reacting components are present in optimal proportions. $X_g + X_b = 14.94$ mm the length of the neutral agar column. D_b was assumed equal to $4.81 \cdot 10^{-7}$ cm²/sec the value for horse tetanus antitoxin, LARGIER[5], and which was confirmed for the precipitin antibodies of Burnupena cincta haemocyanin prepared in the rat, guinea pig and rabbit[6]. Substituting $X_g = 5.14$, $X_b = 9.8$ and $D_b = 4.81 \cdot 10^{-7}$ cm²/sec in eqn. (1) a diffusion constant of $1.32 \cdot 10^{-7}$ cm²/sec at 20° was calculated.

TABLE II

DIFFUSION CONSTANT OF POLIOVIRUS DETERMINED BY THE DIOUBLE GEL DIFFUSION METHOD

Expt. No.	$D \times 10^7$ *cm²/sec*
1	1.29
2	1.26
3	1.32
Average	1.29

In Table II are given the results obtained in three diffusion experiments on poliovirus. The average value, $1.29 \cdot 10^{-7}$ cm^2/sec is probably within $\pm 5\%$ of the true value as judged from previous results of diffusion measurements on proteins by this method[2].

Because of uncertainties regarding the density of the poliovirus particle (SCHAFFER AND SCHWERDT[7]), calculation of the molecular weight was not attempted at this stage.

DISCUSSION

The determination of the diffusion constants of viruses by the usual optical methods is dependent upon the availability of large amounts of pure virus. With few exceptions, notably the plant viruses, such quantities of infective material are usually not available. By applying the double gel diffusion technique of measuring diffusion constants a partial solution to the problem may be obtained. Although the degree of accuracy achieved by the latter method is not as high as that obtained by the optical methods, the relatively small amounts of infective material required for a diffusion experiment may be regarded as compensatory to the sacrifice of greater precision.

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