Estimation of Particle Weights of Myxovirus Antigens by the Gel Diffusion Technique

The presence of several antigenic components in ether-disintegrated preparations of HVJ and NDV has been demonstrated by the agar gel diffusion technique[1,2]; three antigens in both viruses, designated R, H and S, precipitate with viral antiserum. In HVJ, R is a rapidly diffusible component associating with hemagglutinin, H is an inherent hemagglutinating component and S is s-antigen. These antigens have not yet been identified definitely in NDV.

Estimation of the particle weights of these antigens will, doubtlessly, lead to considerable advances in the elucidation of the structure of myxoviruses. We employed the techniques developed by Allison and Humphrey[3,4] to estimate the diffusion coefficients and the particle weights of myxovirus antigens by the gel diffusion method.

Antigens and antiserum were placed in troughs cut rectangulary in a 1 per cent agar layer. The diffusion coefficients (D_{20}) of the antigens were estimated from the formula $\tan\theta=\sqrt{D_b/D_g}$, where D_g and D_b are the diffusion coefficients of the antigen and antibody, respectively, and θ is the angle made between the antigen trough and the straight precipitation line at optimal proportions. Over wide ranges of concentrations of the antigen and antibody, straight precipitation lines were observed. Here D_b, the diffusion coeffiicient of γ-globulin of rabbit serum, is taken as 3.8×10^{-7} $cm^2 \cdot sec^{-1}$ (at 20°C)[5].

Ether-disintegrated preparations of myxovirus and rabbit antisera were prepared as described in the previous reports[1,2]. A typical experiment is shown in Fig. 1, using ether disintegrated HVJ and anti-HVJ rabbit serum.

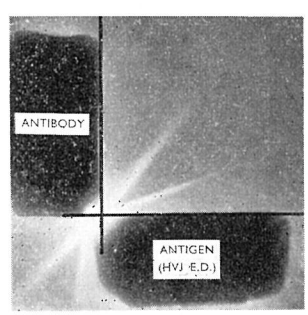

Fig. 1. Photograph of precipitation lines obtained by diffusion of an ether-disintegrated preparation of HVJ and anti-HVJ rabbit serum in 1 per cent agar from troughs cut rectangularly.
A precipitation by R antigen is observed in the antibody side and a precipitation by H antigen, in the antigen side.

The diffusion coefficients of myxovirus antigens thus calculated, are summarized in Table 1, except for antigens which did not precipitate sharply because of their low concentration and the virus particle of NDV which showed no visible diffusion. The particle weights of the myxovirus antigens were calculated from these diffusion coefficients and the corresponding sedimentation coefficients, assuming that the partial specific volume of these antigens is 0.7 and neglecting the hydration volume, and are shown in the Table.

Table 1. Sedimentation and diffusion coefficients and particle weights of myxovirus antigens

Virus	Antigen	Diffusion coefficient	Sedimentation coefficient	Particle Weight
HVJ	R	7.7×10^{-7} cm^2/sec.	4.0 S	3.9×10^4
	H	0.49×10^{-7}		
	V*	0.080×10^{-7}	1200	12×10^8
NDV	R	3.0×10^{-7}	5.4	16×10^4
	S	0.58×10^{-7}		
PR 8	H	0.38×10^{-7}		
	V*	0.19×10^{-7}	700	30×10^7

Particle weights were estimated, using the formula $MW=RTs/(1-V\varrho)D$, where T is the absolute temperature; R, the gas constant; s, the sedimentation coefficient; V, the partial specific volume; ϱ, the density of the suspending medium and D, the diffusion coefficient.

* V indicates the virus particle.

V antigen of HVJ does not exactly correspond to the 1200 S component, as the latter contains R antigen besides V. So the value given for the particle weight of the V antigen of HVJ is not definitive.

The values of diffusion coefficients estimated by this method has a error raange of 10 per cent.

At present, it is not possible to obtain all the sedimenting components coresponding to these antigenic components of myxovirus; both the H and R components of HVJ constitute a hemagglutinin of 90 S[1] and it is not yet certain whether the s-antigen of NDV consists of S alone or both S and R components. Thus the sedimentation coefficients for some components could not be shown in the Table. In further studies on HVJ subunits, the 4.0 S components could be isolated by alkali-treatment of ether disintegrated preparation of HVJ and were identified antigenically with the R component by the gel diffusion technique.

The particle weight given for the Influenza virus, PR8 strain is consistent with results of Schramm[6].

Further studies will be required to determine the position of these antigens in the viral structure.

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