

# Temperature-Dependent Inhibition of Fusion from Without

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Fusion from without by Newcastle disease virus is inhibited by incubation at 23 C. The inhibitory effect is exerted at a step after viral attachment and is not reversed by subsequent incubation at higher temperatures.

Virions of paramyxoviruses, including several strains of Newcastle disease virus (NDV) (2, 11), Sendai virus (12, 13), simian virus 5 (7), and measles virus (4), possess the ability to fuse cells within a short time after infection by high multiplicities of virus. The mechanism of this fusion process, which we have operationally defined as fusion from without (FFWO) (2), is not yet understood. The principal approach in elucidating this mechanism has been to compare the induction of this type of fusion by different virus strains (2, 11), in different cell types (4, 7, 10), by virus stocks grown in different host cells (16), or by virus subjected to a variety of physical and chemical treatments (1, 8, 10). Interpretation of the results of such experiments is complicated by the problem of determining which aspects of high-multiplicity infection are directly involved in the induction of fusion. Our studies on the early interactions of NDV with cell membranes have now revealed a temperature-dependent event which can inhibit the cell-fusing activity of the virion. This finding provides a conditional system involving a single virus-cell combination, which should allow for a more critical evaluation of the relationship between various aspects of virus-cell interaction and the fusion process. In addition, it has important consequences for studies which use virus-induced fusion as a tool (15).

Purified stocks of cloned strains of NDV-N (N. J. LaSota, 1946) and NDV-IM (Italy-Milano, 1945) were prepared by centrifugation of concentrated allantoic fluid-grown stocks (3) through 20% (w/v) sucrose (5). This procedure eliminates those hemagglutinating particles which sediment more slowly than infectious virus and which interfere with FFWO (8). Infectivity and hemagglutination titrations were conducted as previously described (2, 3, 5). Secondary cultures of

chick embryo cells in 60-mm plastic tissue culture plates were used for all fusion experiments. Eagle minimal essential medium, supplemented with 2.5% calf serum and 2.5% Tryptose-phosphate broth and maintained at pH 7.5 to 7.8 (2), was used throughout. Cultures were treated with 0.2 ml of virus (20,000 hemagglutinating units/ml) for 1 hr, and then 5 ml of medium at the designated temperatures was added. Temperature changes were obtained by replacement with medium at the appropriate temperature. After the prescribed incubation periods, cultures were fixed with 95% methanol and stained with Giemsa as previously described (2, 10). The extent of fusion was determined by subtracting the average number of nuclei per cell (previously referred to as the "fusion index" [2, 10]) of uninfected cultures from the average number of nuclei per cell of infected cultures. The value obtained represents the average number of fusion events per cell in the infected cultures (W. R. Gallaher, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1971).

We have previously shown that after adsorption of virus at 4 C, FFWO proceeds rapidly at 38 to 42 C and is essentially complete within 1.5 to 2 hr. Under similar conditions, it proceeds much more slowly at 34 and not at all at 23 C (2). The data in Table 1 suggest that the extent of fusion is also critically affected by the temperature of incubation during the period when virus is adsorbing to the cells. Cultures were treated with virus for 1 hr at various temperatures and then incubated at 42 C for an additional 3 hr. For NDV-N the extent of fusion is considerably less when adsorption is done at 24 rather than at 4 C or at temperatures above 30 C. The extent of inhibition is strain-specific as indicated by the similar, but less dramatic, effect for NDV-IM. Further evidence of this strain specificity is provided by the finding that considerable FFWO can take place after adsorption of NDV-HP (Israel-HP, 1953) at 23 C (2).

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While this study was in progress, Young and Ash reported similar effects of adsorption temperature on the induction of FFWO by still another NDV strain (16). They suggested that this effect was not due to differences in adsorption rates per se, because viral infectivity was removed from the medium equally well at 22 and 37 C. However, recent studies (Clavell and Bratt, *Virology*, *in press*) have raised a question as to whether the same fraction of the virus particle population is being measured in the induction of FFWO as in infection, even though these two properties co-sediment during velocity sedimentation in sucrose (Gallaher and Bratt, *manuscript in preparation*). Therefore, we have confirmed the findings of Young and Ash by using a different approach to distinguish between the effects of temperature on attachment and on events occurring after attachment. After adsorption of virus for 1 hr at 4 C, cultures were either kept at 4 C or shifted to other temperatures for an additional hour. All cultures were then shifted to 42 C for another 2 hr. Table 2 shows that incubation at temperatures below 37 C (particularly 23 C) during the first hour after attachment greatly reduces the extent of FFWO seen after subsequent incubation at 42 C. It is therefore apparent that subsequent to attachment, a virus-cell interaction occurs at these intermediate temperatures which inhibits the induction of

FFWO. This inhibitory effect is not reversed by subsequent incubation at 42 C.

We next analyzed the kinetics of this inhibitory effect in the temperature-shift experiment shown in Fig. 1. After adsorption of NDV-N at 4 C, cultures were incubated at either 23 or 42 C. At subsequent times cultures were either shifted up from 23 to 42 C, or down from 42 to 23 C. All cultures were incubated at the second temperature until a total of 3 hr after adsorption. The descending curve, which shows the extent of fusion in cultures shifted from 23 to 42 C, reveals that initial incubation at 23 C for 30 min almost completely prevents FFWO, despite subsequent incubation for 2.5 hr at 42 C. On the other hand, the curve for the cultures shifted down from 42 to 23 C shows that as little as 20 min at 42 C allows considerable fusion to take place despite subsequent incubation for 2.5 hr at the nonpermissive temperature. It seems likely that most of the fusion found under these conditions occurs during the 20 min at 42 C since other studies have shown that FFWO becomes insensitive to the inhibitory effect of antiviral antibody within 20 min at 42 C (2) and membrane fusion can be observed by this time at 37 C (9). The reciprocal effects of incuba-

TABLE 1. Effect of adsorption temperature on the induction of FFWO

Adsorption temp (C)	Fusion events per cell	
	NDV-N	NDV-IM
4	1.68	0.54
24	0.15	0.34
30	0.94	0.49
35	0.83	0.71
37	0.88	0.75
42	1.09	0.78

TABLE 2. Effect of postadsorption temperature on the induction of FFWO

Temp during first hr after adsorption at 4 C	Fusion events per cell after 2 additional hr at 42 C	
	NDV-N	NDV-IM
4	0.59	0.39
24	0.07	0.10
30	0.48	0.32
37	0.79	0.48
42	1.19	0.73

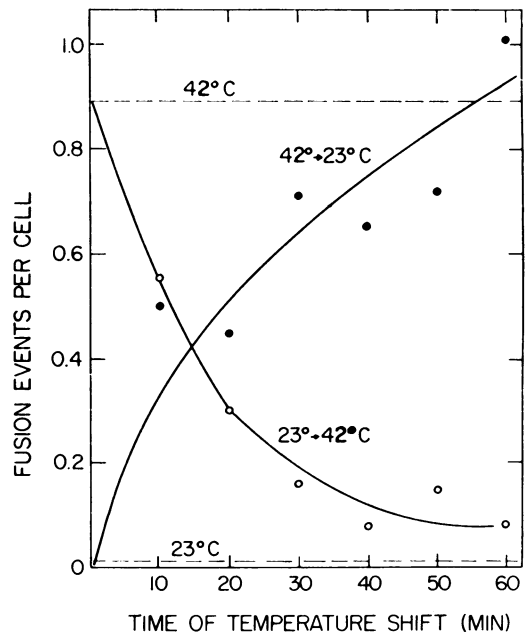


FIG. 1. Kinetics of the inhibition of FFWO by incubation at 23 C. Symbols: ○, extent of fusion in cultures shifted from 23 to 42 C; ●, extent of fusion in cultures shifted from 42 to 23 C. The dashed lines indicate the extent of fusion in cultures incubated at 23 or 42 C for 3 hr.

tion, at 23 and 42 C during the initial period after viral adsorption, suggest that virus particles and cells irreversibly interact, either in a manner which is conducive to fusion or in one which is not conducive to it.

The mechanism of this inhibition of FFWO is, like the mechanism of FFWO itself, not understood, but preliminary results from our laboratory suggest the inhibition is not due to more rapid elution of virus at 23 C. In addition, because, within the time course of these experiments, virus-treated cells shifted from 23 to 42 C are indistinguishable from uninfected cultures, the inhibition of FFWO obtained during this procedure is not due to cell damage as it is in the case of the inhibition of fusion at low calcium ion concentrations (12).

Although its mechanism is unknown, this temperature-dependent inhibition of FFWO may have important practical implications for cell hybridization studies. Where maximal fusion at the lowest possible virus concentration is desired, adsorption of virus at 23 C should be avoided, unless fusion by the inducing virus strain has been shown to be unaffected by incubation at this temperature.

These techniques should also be of use in analyzing the mechanism of FFWO since variations in the virus-cell interaction can be studied under conditions where the same multiplicity of the same strain of virus has interacted with all cultures. The effect of temperature shifts during the first 30 min of infection constitutes the only known conditional system for inducing variations of FFWO. This conditional system may serve as a critical test for determining the relationship between the induction of FFWO and other effects of high multiplicity infection, such as the activation of lysosomes (14) or the inhibition of lipid synthesis (W. R. Gallaher and H. A. Blough, *Bacteriol. Proc.*, p. 215, 1972).

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