THE NEURAMINIDASE OF MEASLES VIRUS

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The agent of measles (rubeola) has been assumed to be a paramyxovirus on the basis of its electron microscopic appearance, its size, its sensitivity to ether and detergents, and the presence of hemagglutinin in the viral envelope. Thus far, however, the one attribute common to myxoviruses, namely neuraminidase, has not been described for measles virus. We have demonstrated that specific neuraminidase activity is associated with measles viral hemagglutinin. The evidence suggests strongly that the enzyme is part of the viral envelope.

MATERIALS AND METHODS

Measles virus (Edmonston strain) was propagated in HeLa or amnion cell monolayers grown in 32 oz prescription bottles. After adsorption of appropriately diluted virus onto washed monolayers for 1 hr at 37°C, cells were maintained for 6 to 7 days in Eagle's basal medium supplemented with 0.1% lact-albumin hydrolysate (LH) without serum. Cells were then scraped into the fluid and concentrated by centrifugation at 1500 X g; the pellet was frozen and thawed successively three times in a small volume of culture fluid, which, after

low speed clarification, was added to the remaining volume of culture fluid. The whole was then centrifuged at 33,000 X g for 2 hr at 4°C in a Spinco model L ultracentrifuge. The pelleted virus was taken up in phosphate-buffered saline, pH 7.2, to effect a 10- to 20-fold concentration of hemagglutinin (HA) and infectivity.

Hemagglutination-inhibition tests were performed by the pattern technique with 2% rhesus cells, using four hemagglutinating units (HAU) of virus. Infectivity titrations were performed by inoculating decimal dilutions of virus onto replicate amnion cell monolayers. After 3 days' incubation at 37°C under serum-free medium, cells were examined for hemadsorption of rhesus erythrocytes (0.5%). Neuraminidase activity was determined in 0.1 ml of concentrated virus added to an equal volume of substrate [(Collocalia mucoid (Howe et al. 1961), neuraminlactose (NAL) (Calbiochem), or urinary mucoprotein (UMP)] in pH 5.5 phosphate buffer. After incubation at 37°C for 16 to 18 hr, free N-acetylneuraminic acid (NANA) was measured by the thiobarbituric acid method (Warren, 1959). Controls (virus alone, substrate alone, and homogenates of normal cells) gave negligible color values. Free NANA was identified by descending chromatography of the reaction mixtures on Whatman no. 1 paper using isobutyric acid-water(5:3), adjusted to pH 3.7 with concentrated NH_4OH, as solvent (Magasanik et al. 1950). Spots were developed by the thiobarbituric acid reaction (Warren, 1960), with crystalline NANA as reference standard. Absorption curves were plotted with a Cary model 11M recording spectrophotometer.

RESULTS AND DISCUSSION

Incubation of concentrated measles virus (64-640 HAU)

TABLE I

Experiment No.	Cell line	HAU virus added[a]	µg Substrate[b] Collocalia mucoid	NAL	nM NANA liberated[c]
1	HeLa	640	500		29
2	Amnion	320	200		17
				25	23
3	Amnion	640	200		28[d]
				100	75[d]
4	HeLa	64	UMP[e] 202		17

[a] Gave negligible color value in absence of substrate.

[b] Gave negligible color value in absence of virus.

[c] 37°C for 18 hr.

[d] Descending chromatogram gave single spot with Rf of crystalline NANA.

[e] Urinary Mucoprotein: 110µg inhibited 4 HAU.

with either Collocalia mucoid or neuraminlactose at pH 5.5 at 37°C resulted in the liberation of thiobarbituric acid chromophore (Table I). Increasing the amount of substrate with a constant amount of virus resulted in correspondingly increased chromophore formation. The chromophore was identified as NANA by its characteristic absorption peak at 549 mµ and by descending paper chromatography, which showed its

mobility to be the same as that of crystalline NANA. Urinary mucoprotein (UMP), a potent inhibitor of myxovirus hemagglutinin, was also susceptible to the viral enzyme. The neuraminidases of other paramyxoviruses (Darrell and Howe, 1964; Tosawa et al. 1967) appear to be strain specific. Serologic analysis of measles virus neuraminidase is in progress. The foregoing results constitute the first reported demonstration that measles virus possesses a neuraminidase and further justify its designation as a paramyxovirus.

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