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# Hemadsorption Cell-Counting Assay of Interferon

*Brief Report*

By

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With 1 Figure

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Although a wide variety of procedures have been described to assess interferon activity (4), efforts continue towards developing assay systems that embody the desirable attributes of minimal complexity, maximal sensitivity, reliability, and rapidity (1, 10, 11, 12). A simple assay for interferon was evolved that is based on the enumeration of individual cells manifesting hemadsorption within 20 to 24 hours of infection. Two previously reported interferon assays, that of quantitative hemadsorption (3) and immunofluorescent cell-counting (7), were combined to develop the hemadsorption cell-counting assay of interferon described in this report.

A stock pool of parainfluenza 1 (Sendai) virus, consisting of allantoic fluids obtained from virus-inoculated 11-day-old embryonated chicken eggs, was used to challenge interferon-treated clone 1-5C-4 human cell monolayers (13). Both virus and cells were obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. Cells were propagated with Eagle's minimum essential medium (MEM) containing 10 per cent fetal calf serum (FCS) and maintained with MEM plus 5 per cent FCS. For interferon assays, cells were cultivated on glass cover slips (15-mm diameter) inserted in flat-bottomed glass vials (19 by 65 mm).

Human leukocytes treated with ultraviolet-irradiated PR8 influenza virus provided the source of interferon preparations employed in this study. The procedure for interferon induction was similar to that reported with another system (8). The preparation possessed the biological and physical properties ascribed to viral interferons (9).

To assay interferon, serial twofold dilutions of interferon prepared in maintenance medium were introduced in 1.0 ml volume directly into vials containing cover slip cell monolayers and incubated at 35° C for 20 to 24 hours. Assays were carried out in duplicate. After incubation, cell monolayers were washed with phosphate-buffered saline (PBS), pH 7.1, and then challenged with virus in 0.2 ml volume. Virus was diluted to give from 1 to 3 infected cells per microscopic field.

Controls consisted of untreated cell monolayers infected with virus. Challenge virus was attached to cells by either centrifugal force (6) or incubation at 35° C for 1 hour. After the attachment period, 1.0 ml of maintenance medium was added to vials which were then incubated at 35° C for approximately 22 hours. Cover slip cell monolayers were then rinsed with PBS. A 0.4 per cent suspension of guinea pig erythrocytes in PBS was added in 0.5 ml volume onto each cell monolayer which was held at 6°C for 20 minutes (5). Cell monolayers were then rinsed twice with PBS.

For enumerating cells exhibiting hemadsorption, cover slip cell monolayers were examined with a Nikon-inverted microscope, magnification of ×200. Thirty microscopic fields were examined on each cover slip. The reciprocal of the interferon dilution that reduced the number of infected cells by 50 per cent of the virus control served as the measure of interferon activity, *i.e.*, 50 per cent $ICDD_{50}$ (50 per cent infected cell-depressing dilution). This value was interpolated by plotting on logarithmic probability paper the reduction percentages against the logarithm of corresponding interferon dilutions. With this system, 0.7 international reference human (69/19) interferon units assayed as one unit.

The immunofluorescence assay of Sendai virus was based on counts of fluorescent clone 1-5C-4 cells in infected monolayers that had been stained with viral antiserum conjugated with fluorescein isothiocyanate. Details of antiserum conjugate preparation, staining, microscopic examination, and infected-cell enumeration have been described elsewhere (6).

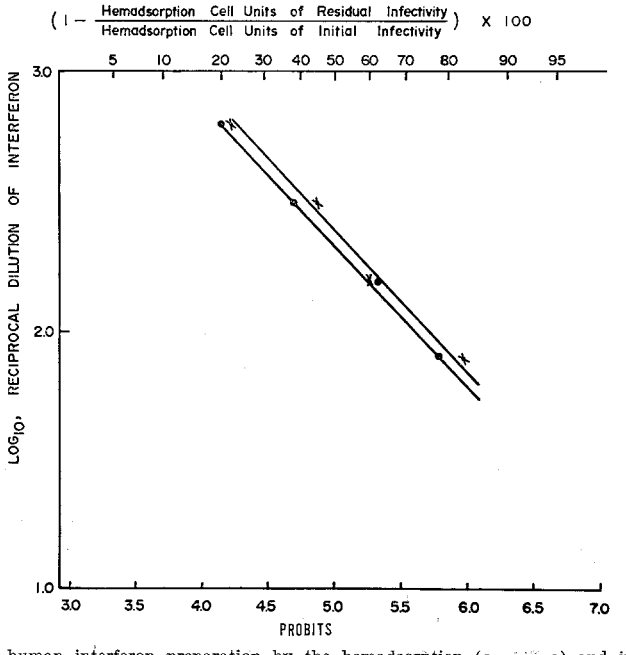


Fig. 1. Assay of human interferon preparation by the hemadsorption (●——●) and immunofluorescent (×——×) cell-counting procedures employing clone 1-5C-4 cell monolayers challenged by Sendai virus

Results of a comparison in sensitivity between the hemadsorption and immunofluorescent cell-counting assays of the same interferon preparation are shown in Figure 1. Equivalent interferon titers and parallel dose-responses were obtained

with both assays. When Sendai virus-challenge doses, differing tenfold in concentration, were employed in the hemadsorption assay, there was no significant change in interferon titer. That the titer of an interferon preparation is independent of the virus-challenge dose agrees with previous findings (3, 7).

To estimate the precision of the assay, ten replicate determinations of the same interferon preparation were made. Titers ranged from 400 to 470 units/ml with a mean of 439 units. The standard deviation was $\pm 29.2$; the coefficient of variation was 6.6 per cent. The precision of the assay compared favorably to that reported for other methods (4). The reproducibility of the interferon assay is shown in Table 1. Interferon titers, *i.e.*, 205, 250, 225, 220, of the same preparation determined at weekly intervals showed less than twofold differences.

Table 1. *Reproducibility of Hemadsorption Cell-Counting Assay of Interferon*

| Week | Interferon Dilutions | | | | Interferon $ICDD_{50}$/ml [b] |
|---|---|---|---|---|---|
| | 1:80 | 1:160 | 1:320 | 1:640 | |
| 1 | 77 [a] | 57 | 39 | 19 | 205 |
| 2 | 78 | 63 | 38 | 32 | 250 |
| 3 | 83 | 60 | 45 | 17 | 225 |
| 4 | 80 | 58 | 38 | 22 | 220 |

[a] Percentage reduction of Sendai virus-infected cell counts.
[b] Reciprocal of 50 per cent infected cell-depressing dilution.

The time required for interferon to impart maximal cell resistance to infection was determined in an attempt to shorten the time for completion of the assay. Maximal interferon titers were obtained when cells were treated with interferon from 20 to 24 hours before the addition of challenge virus. Interferon titers were three- and twofold less at 4 and 8 hours, respectively. While resistance conferred to human cells by human interferon requires approximately 18 hours (2), cells of other animal species may develop resistance to infection within 8 hours of exposure to species specific interferon. It is possible, therefore, to complete the assay of certain interferons by the hemadsorption cell-counting procedure within 36 hours. The simplicity, rapidity, high precision, and reproducibility of this procedure adequately fulfills the proposed criteria for interferon assessment (4).

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