INFECTION AND IMMUNITY, June 1976, p. 1626-1632
Vol. 13, No. 6

Printed in U.S.A.

Rapid Quantitation of Interferon with Chronically Oncornavirus-Producing Cells

MORDECHAI ABOUD, OFRA WEISS, AND SAMUEL SALZBERG*
Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

Received for publication 25 February 1976

The capacity of interferon to inhibit virus production in cells chronically infected with oncornavirus enabled us to develop a simple system for interferon quantitation that was independent of exogenous viral infection. The release of the virus to the culture medium was determined by its reverse transcriptase activity. The inhibitory effect of interferon in this system was linearly proportional to the log of its dilution over a range between 5 and 80% inhibiton. The sensitivity of the system was comparable to that of the vesicular stomatitis virus plaque reduction assay, whereas its reproducibility was found to be even better. This method is very rapid and can be completed within less than 24 h.

Interferon (IF) can be quantitatively assayed by a variety of methods. Most of them rely on the potency of IF to render cell cultures resistant to the consequences of viral infection such as plaque formation (6, 9, 10, 12, 20, 30), cytopathogenic effect (2, 7, 9-12, 25), virus release (10, 12), viral nucleic acid synthesis (1, 24), hemadsorption (8, 10, 12), and neuraminidase production (27, 28). A virus-free system based on the ability of IF to increase the sensitivity of cells to the cytopathic effect of poly(rI)·poly(rC) has also been described (30). An ideal method for IF quantitation would be one that is sensitive, accurate, and reproducible but at the same time rapid and simple to perform and score. None of the available methods can thus be regarded as perfectly satisfying, and a further improvement is, therefore, still required (see reviews by Finter [10, 12]).

In all virus-dependent IF assays known so far, cells are treated for several hours with IF and then infected by the challenging virus. Since the consequences of the viral growth can usually be detected in cell cultures only after 1 to 3 days, the inhibitory effect of IF cannot be determined earlier. It is, therefore, obvious that, if the need for virus infection after the treatment with IF could be avoided, the assay of IF would be substantially simplified and abbreviated. Such a convenient system became available after the discovery of the inhibitory effect of IF on the release of oncornaviruses by chronically infected cells (3-5, 13, 14, 19). The effect of IF can thus directly be detected in these cells without prior infection by exogenous virus. Furthermore, reverse transcriptase activity characteristic of oncornaviruses (31) provides an easy quantitation of these viruses (26). Utilizing the outstanding advantages of this system, we have developed a rapid and simple assay for mouse IF that is highly reproducible and accurate. This assay is as sensitive as the most commonly used vesicular stomatitis virus (VSV) plaque-forming reduction assay (12).

MATERIALS AND METHODS

Cells and viruses. L_{929} cells were used for IF production and plaque assay.

NIH/3T3 cells chronically infected with the Moloney strain of murine leukemia virus (M-MLV) were used for developing the new method of IF titration.

The cells were grown in Dulbecco modified Eagle medium (DMEM) containing either 2 or 10% heat-inactivated calf serum as indicated in each case and incubated at 37 C in a humidified incubator supplemented with 5 to 10% CO_2.

Newcastle disease virus (NDV) Herts strain was propagated in the allantoic fluid of 11-day-old fertilized chicken eggs, titrated by plaque formation on monolayers of primary cultures of 11-day-old chicken embryo cells, and used for induction of IF. VSV was propagated on L_{929} cells and used for plaque assays.

IF production and partial purification. Confluent monlayers of L_{929} cells were infected for 1 h with NDV at a multiplicity of 7 plaque-forming units/cell. The cells were then washed three times with phosphate-buffered saline (PBS) (26) and were provided with serum-free DMEM. After 16 to 18 h, the medium from the infected cultures was pooled. Mock-infected cultures were used as controls. One sample of the pooled medium was desginated IF-A. The rest was acidified with HCl to pH 2, and a sample was withdrawn, kept at 4 C for 6 days, and then neutralized with NaOH. This sample will be referred to as IF-B. The remaining acidified medium was concentrated eightfold by vacuum dialysis as described by Falcoff et al. (7). This procedure lasted for 3 to 4 days. The concentrated material was kept

at 4 C for an additional 2 to 3 days. The large precipitate appearing in this stage was removed by centrifugation. The clear fluid was neutralized by NaOH, resulting in another heavy precipitate that was also cleared out. This supernatant will be referred to as IF-C. Part of IF-C was fractionated by ammonium sulfate as described by Knight (18). The supposedly IF-containing fraction precipitating at 40 to 65% ammonium sulfate saturation was dissolved in 0.2 volume of 0.1 M phosphate buffer, pH 6, and dialyzed against the same buffer. This will be designated IF-D. All IF preparations were centrifuged for 2 h at 60,000 × *g* to remove residual NDV and were kept in small portions at −70 C.

Mouse serum IF. Mouse serum IF was kindly provided by R. Apte of our department. It was prepared by intravenous injection of 100 μg of poly(rI)·poly(rC) per mouse, and bleeding was done after 3 h.

IF titration by reverse transcriptase reduction assay. Approximately 8 × 10^5 NIH/3T3 M-MLV cells were plated per tissue culture dish (50 mm) in 5 ml of DMEM containing 10% calf serum. After 24 h, the medium was removed and 2 ml of serial 0.3 $\log_{10}$ IF dilutions (serial twofold dilutions) in DMEM containing 2% calf serum was added to duplicate cultures. Cultures receiving medium without IF served as the control. After an additional 16 to 18 h, cultures were washed three times with PBS and supplemented with 2 ml of DMEM containing 2% calf serum. After 3 to 4 h, the cultures were brought to 4 C and the culture fluid was collected for reverse transcriptase assay. The fluids were centrifuged at a low speed to remove cellular debris, and 50-μl samples were used for reverse transcriptase assay, as was essentially described elsewhere (26). The reaction mixture (final volume, 100 μl) contained 50 mM tris(hydroxymethyl)aminomethane - hydrochloride, pH 8.2, 5 mM dithiothreitol, 100 mM NaCl, 0.5 mM $MnCl_2$, 0.03% Triton X-100, 50 μCi of [^{3}H]thymidine methyl triphosphate per ml (10 Ci/mmol), and 10 μg of poly(A)·oligo(dT) per ml as an exogenous template. After incubation for the indicated time at 37 C, 50-μl aliquots were spotted on Whatman DE 81 cellulose disks. The disks were collectively washed five times with 5% (wt/vol) Na_2HPO_4 (5 ml/disk, 4 min each time) and once more with distilled water, dried, and counted in scintillation fluid. Reverse transcriptase reduction dose unit is defined as that dose of IF which reduces reverse transcriptase activity by 50%.

IF titration by VSV plaque reduction assay. IF titration by VSV plaque reduction assay was performed as described by Stewart et al. (30) with the same dilutions used for reverse transcriptase reduction assay.

Protein determination. Protein determination was done by the method of Lowry et al. (21).

RESULTS

Reverse transcriptase reaction. In preliminary experiments we followed the procedure of others (14, 19, 26) and concentrated the virus released to the medium of NIH/3T3 M-MLV cultures by high-speed centrifugation and resuspension in a small volume before analyzing its reverse transcriptase activity. But this time-consuming process was inadequate for a rapid assay of many samples included in IF titration. In attempts to simplify the procedure, we found the enzyme activity present in the medium sufficiently high, even without concentrating the virus. Hence, in all the following experiments, reverse transcriptase was measured in the culture medium directly after low-speed centrifugation for removal of cellular debris. In the experiment illustrated in Fig. 1A, the reaction measuring the enzyme activity proceeded at a linear rate for at least 60 min. To use the enzyme activity as a measure for virus concentration, it was first necessary to determine whether this activity was linearly proportional to the amount of virus-containing fluid added to the reaction. This, indeed, was the case in the periment illustrated in Fig. 1B. Furthermore, the two observations indicate the absence of inhibitory quantities of antagonists, such as nucleases or phosphatases, which might have progressively interfered with the ribonucleic acid-directed deoxyribonucleic acid synthesis.

Effect of IF on virus production by NIH/3T3 M-MLV cells. To demonstrate the effect of IF on the virus production by NIH/3T3 M-MLV cells, the virus release, as measured by reverse transcriptase, was followed in IF-pretreated and untreated cells. After 16 to 18 h of treatment with IF-B (see above), the cultures were

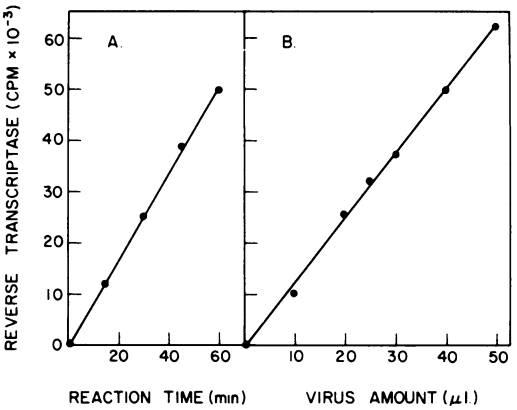

FIG. 1. *Kinetics of the reverse transcriptase reaction. About 24 h after plating NIH/3T3 M-MLV cells, the medium was changed, and 14 h later the medium was collected and centrifuged at 800 × g to remove cell debris. Enzyme activity was measured (A) as a function of the reaction time (using 50-μl aliquots of the medium) or (B) as a function of the amount of the medium added to the reaction (using a 45-min reaction of time). The total volume was 100 μl. CPM, Counts per minute.*

rinsed three times with PBS, and 2 ml of DMEM containing 2% calf serum was added. As shown in Fig. 2, the virus accumulated in the medium at a linear rate for at least 6 h. Pretreatment with IF-B at a dilution of 1:16 reduced this rate to about 30% of the untreated control, whereas a twofold increase in IF concentration reduced the virus release to about 15%. The possibility that the inhibition in the virus release resulted from a general cytotoxic effect of the IF preparation was ruled out by the experiment illustrated in Fig. 3. In this experiment we observed no effect on viability of cells, even when incubated with a higher concentration of IF-B (at 1:3 dilution). Furthermore, no effect of IF pretreatment was observed on the incorporation of labeled deoxythymidine, uridine, and amino acid into acid-insoluble material (unpublished data). The linear rate of the virus release makes it possible to collect the fluids from the tested cultures for enzyme assay

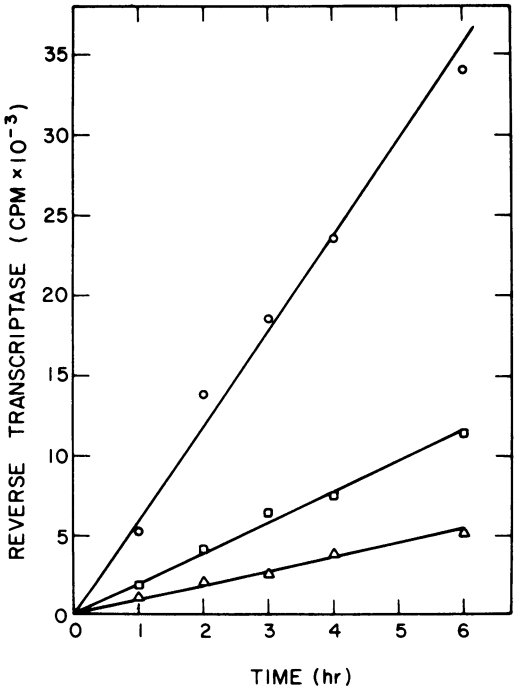

FIG. 2. *Effect of IF on virus production by NIH/3T3 M-MLV cells. One series of cultures received IF-B diluted 1:16 in DMEM containing 2% calf serum (□); another received IF-B diluted 1:8 (△), whereas a third series, serving as a control (○), received no IF in the medium. After 16 to 18 h, the cultures were rinsed three times with PBS, and 2 ml of DMEM containing 2% calf serum was added. Reverse transcriptase activity in the medium was determined at the indicated times thereafter. CPM, Counts per minute.*

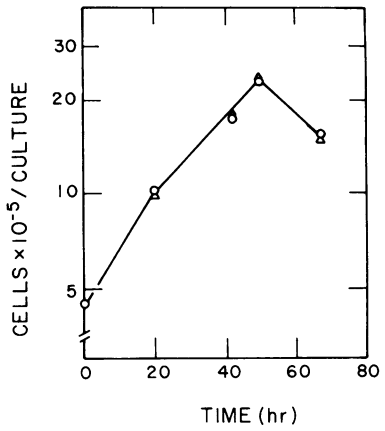

FIG. 3. *Effect of IF on viability of NIH/3T3 M-MLV cells. The medium was removed 24 h after plating, and 2 ml of DMEM containing 2% calf serum was added to the cultures with (△) or without (○) 1:3 dilution of IF-B. At the indicated times thereafter, the cells were counted in both treated and untreated cultures.*

at any convenient time during the first 6 h after IF treatment.

IF titration by reverse transcriptase reduction and VSV plaque reduction assays. IF produced by the NDV-induced L_{929} cells was titrated at its various stages of purification by the reverse transcriptase reduction method and, in parallel, by the VSV plaque reduction method. A 2-ml amount of serial 0.3 $\log_{10}$ IF dilutions in DMEM plus 2% calf serum was added to duplicate cultures of NIH/3T3 M-MLV cells and of L_{929} cells. After 16 to 18 h, the cultures were washed three times with PBS. NIH/3T3 M-MLV cells received 2 ml of DMEM containing 2% calf serum and were incubated for 3 to 4 h before medium was collected for enzyme assay. L_{929} cells were infected with 100 to 200 plaque-forming units of VSV, and plaques were counted 48 h later. Figure 4 shows that the titers of IF from all stages of purification were similar in both methods, indicating that the plaque inhibitory activity was copurified with the reverse transcriptase reducing activity. The closed titers also indicate that the sensitivity of both methods is comparable. To determine the reproducibility of the methods, the titration of IF-A was repeated three times, with no significant variation in the reverse transcriptase reduction assay, whereas a variation extending up to twofold was observed with the plaque reduction assay (Fig. 4A). The inhibition in the reverse transcriptase activity was linearly proportional to the log of IF dilution, ranging between 20 and 95% of the control activity.

Table 1 summarizes the titers and the specific activities of the various IF preparations, as computed from the reverse transcriptase data presented in Fig. 4 and the protein determination of these preparations. IF-A contained 64 U/ml and IF-B contained 70 U/ml, indicating no loss of IF activity due to pH 2 treatment for 6 days at 4 C. IF-C is the product of an eightfold concentration by vacuum dialysis, and it contained 560 U/ml, an eightfold increase over IF-B, indicating no loss of IF activity during this process either. The large precipitates that appeared after this step contained no IF activity, thus resulting in a threefold increase in the specific activity of the cleared supernatant. IF-D is that fraction of IF-C precipitating at 40 to 65% ammonium sulfate saturation. No additional purification over IF-C was achieved by this step. This is consistent with the observation of Knight (18) that in a neutral pH IF precipitates over a broad range of ammonium sulfate saturation with no considerable purification.

Figure 5 shows the titration curves of IF present in mouse serum after poly(rI)·poly(rC) injection. The titer obtained by the VSV plaque reduction in this particular experiment appeared to be about twice that obtained by the

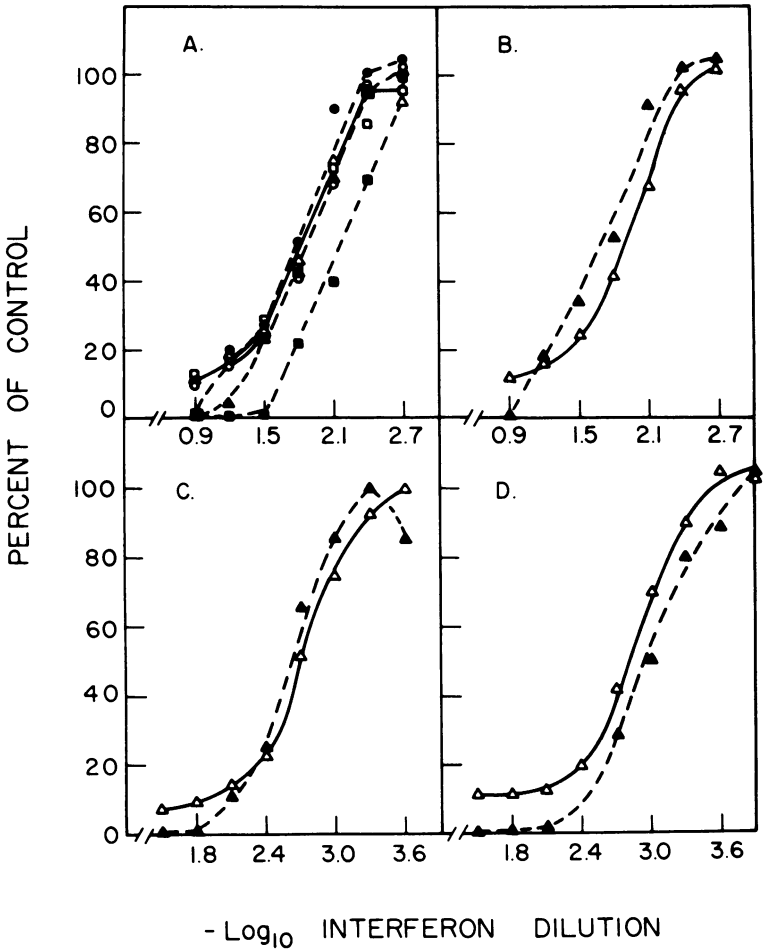

FIG. 4. *Titration of IF at various stages of purification by reverse transcriptase reduction and VSV plaque reduction assays. Duplicate cultures of NIH/3T3 M-MLV and L_{929} cells were treated with serial IF dilutions for 16 to 18 h. Control cultures were treated with IF-free medium. NIH/3T3 M-MLV cultures served for reverse transcriptase reduction assay (open symbols), and L_{929} cultures served for VSV plaque reduction assay (closed symbols). (A) IF-A. With this preparation, titration by both methods was repeated three times: experiment 1 (△ and ▲), control values were 23,580 counts/min and 176 plaques; experiment 2 (○ and ●), 31,760 counts/min and 215 plaques; experiment 3 (□ and ■), 18,900 counts/min and 173 plaques. (B) IF-B, 18,870 counts/min and 115 plaques; (C) IF-C, 25,000 counts/min and 196 plaques; (D) IF-D, 22,070 counts/min and 136 plaques.*

TABLE 1. *IF activity at various stages of purification*

IF[a]	IF activity	
	$RTRD_{50}$ units/ml of protein[b]	$RTRD_{50}$ units/mg of protein
A	64	1,020
B	70	1,176
C	560	3,396
D	680	3,122

[a] See text for IF purification.
[b] $RTRD_{50}$, Reverse transcriptase reduction dose.

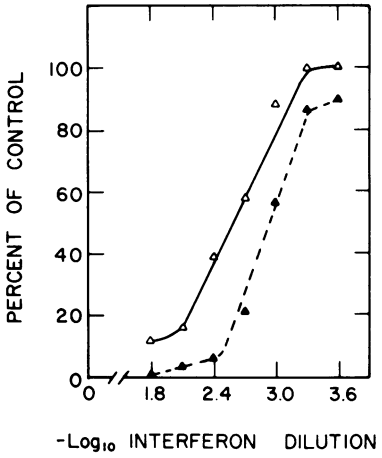

FIG. 5. *Titration of serum IF. This serum was titrated by reverse transcriptase reduction assay (△) and by plaque reduction assay (▲). Control values were 23,280 counts/min and 104 plaques.*

reverse transcriptase reduction assay. Nevertheless, since variations up to twofold were observed in repeated experiments with the plaque assay (Fig. 4A), we consider this difference between the two methods insignificant.

DISCUSSION

The vital importance of IF has led many laboratories to focus their efforts on developing and improving methods for its quantitation (1, 2, 6–12, 15–17, 20, 24–27, 30, 32). However, none of the numerous reported methods proved to be completely satisfactory (12). Some are laborious and time consuming, whereas others are inaccurate, insensitive, or unreproducible. A further improvement is, therefore, essential.

IF induces in cells an antiviral state that inhibits the replication of most ribonucleic acid and deoxyribonucleic acid viruses (12, 29). The development of this state requires several hours (29). Hence, IF treatment is usually inefficient if viral infection occurs before the antiviral state has been fully expressed (12). However, the production of ribonucleic acid tumor viruses in chronically infected cells is blocked by IF, even though it is added many generations after infection (3–5, 13, 14, 19). It appeared to us that such cells might actually provide a system in which the effect of IF could be directly detected, with no need for exogenous viral infection after the treatment with IF. The elimination of the exogenous infection would greatly simplify the assay of IF activity. Moreover, the easy quantitation of oncornaviruses by determining their reverse transcriptase activity (26) makes such a system even more appealing for this purpose.

In the course of characterizing this system, we found a linear increase of reverse transcriptase activity in the culture medium occurring for at least 6 h. The rate of this increase was reduced by pretreatment with IF. McCormick et al. (23) have shown that the reverse transcriptase activity can be inhibited by various agents, such as ribonuclease, present in the virus-containing materials, and thus it cannot be correlated directly with the number of viral particles. The linearity we found in the enzyme activity, with respect to the reaction time and to the amount of the virus-containing fluid, ruled out the existence of inhibitory quantities of such agents in our system. This, together with the correlation found by Friedman and Ramseur (14) between the reductions in the enzyme level and the number of the infective particles in IF-treated cultures, strongly suggested that the reduction in the enzyme activity faithfully reflected an inhibition of the virus release. No effect was observed on the viability of the cells, even when treated with a relatively high concentration of IF, thus ruling out the possibility that cytotoxicity of the preparation was involved in the interference with the virus production. The extent of the inhibition was proportional to IF concentration, providing a useful system for its accurate quantitation. The linear rate of virus release makes this assay very convenient, since it permits the measurement of the enzyme activity at any time during at least the first 6 h after IF treatment. The enzyme assay itself is also flexible and can be adjusted to the convenience of the worker due to the linearity of the reaction observed during at least 60 min.

We used this system for titration of IF from two different sources and at various purification stages. The virus release, measured by reverse transcriptase activity, responded linearly to the log of IF dilution, ranging between 5 and 80% inhibition. To evaluate the sensitivity of this method we compared the titration

curves obtained by the reverse transcriptase reduction assay to those obtained by the VSV plaque reduction assay and found them to be similar. The reverse transcriptase reduction method is, therefore, as sensitive as the most conventional method. Furthermore, in our hands it is also more reproducible, as indicated by three repeated experiments with the same IF preparation. The titration curves with the reverse transcriptase reduction assay were practically identical, whereas a variation extending up to twofold was observed with the plaque reduction method.

In all the experiments reported here, IF was applied 24 h after plating the cells. Hence, the whole procedure lasts less than 48 h. The VSV plaque reduction assay, on the other hand, requires a total time of at least 4 days, since plaques can be counted only 2 days after infection. It is worthwhile to note that we found it possible to shorten the reverse transcriptase reduction assay even further by plating the cells in a medium already containing IF. Titers obtained by this modification were identical to those obtained when IF was added 24 h after plating, whereas the total time of the procedure is reduced to less than 24 h (unpublished data). In principle this modification can be applied also to the plaque assay. However, such a procedure subjects the assay of the plaque to a severe technical problem. Since IF effect is more efficiently pronounced in cells grown with a low concentration of serum in the medium (14, 29), we treated the cells with IF in the presence of only 2% calf serum. Under these conditions L_{929} cells looked much smaller than with 10% calf serum, and confluent cultures were obtained only when extremely large numbers of cells were plated. The efficiency of plaque formation in such cultures was inconsistent and, therefore, more than duplicate cultures for each IF dilution were required to obtain reliable results. Such difficulties were not experienced with the reverse transcriptase method, since this assay was not affected by the shape of the cells in culture.

In summary, we feel that the reverse transcriptase reduction assay offers a great improvement compared with most available methods. It is very rapid and can be completed within less than 24 h. Its performance is simple and flexible, and its sensitivity, accuracy, and reproducibility were found to be highly satisfactory.

Although the described method was developed for mouse IF, it can be applied for other species of IF, particularly for human IF, since human cells releasing oncornaviruses are available (22).

ACKNOWLEDGMENTS

This investigation was supported by a grant (Biotechnology no. 13) from the German Federal Ministry of Research and Technology and by grant no. 01225/8 from the Research Authority, Bar-Ilan University.

LITERATURE CITED

1. **Allen, P. T., and D. J. Giron.** 1970. Rapid sensitive assay for interferons based on the inhibition of MM virus nucleic acid synthesis. Appl. Microbiol. 20:317–237.
2. **Armstrong, J. A.** 1971. Semi-micro, dye-binding assay for rabbit interferon. Appl. Microbiol. 21:723–725.
3. **Billiau, A., V. G. Edy, E. De Clercq, H. Heremans, and P. De Somer.** 1975. Influence of interferon on the synthesis of virus particles in oncornavirus carrier cell lines. III. Survey of effects on A-B- and C-type oncornaviruses. Int. J. Cancer 15:947–953.
4. **Billiau, A., V. G. Edy, H. Sobis, and P. De Somer.** 1974. Influence of interferon on virus particle synthesis in oncornavirus-carrier lines. II. Evidence for a direct effect on particle release. Int. J. Cancer 14: 335–340.
5. **Billiau, A., H. Sobis, and P. De Somer.** 1973. Influence of interferon on virus particle formation in different oncornavirus carrier lines. Int. J. Cancer 12:646–653.
6. **Campbell, J. B., T. Grunberger, M. A. Kochman, and S. L. White.** 1975. A microplaque reduction assay for human and mouse interferon. Can. J. Microbiol. 21:2147–1253.
7. **Falcoff, E., R. Falcoff, B. Lebleu, and M. Revel.** 1973. Correlation between the antiviral effect of interferon treatment and inhibition of in vitro mRNA translation in noninfected L cells. J. Virol. 12:421–430.
8. **Finter, N. B.** 1964. Quantitative haemadsorption, a new assay technique; assay of interferon. Virology 24:589–597.
9. **Finter, N. B.** 1966. Interferon assays and standards, p. 87–118. *In* N. B. Finter (ed.), Interferons. W. B. Saunders Co., Philadelphia, Pa.
10. **Finter, N. B.** 1968. Interferon assays: sensitivity and other aspects, p. 203–212. *In* G. Rita (ed.), The interferons. Academic Press Inc., New York.
11. **Finter, N. B.** 1969. Die uptake method for assaying viral cytopathogenicity and its application to interferon assay. J. Gen. Virol. 5:419–427.
12. **Finter, N. B.** 1973. The assay and standardization of interferon and interferon inducers, p. 135–169. *In* N. B. Finter (ed.), Interferons and interferon inducers. North Holland Publishing Co., Amsterdam.
13. **Friedman, R. M., E. H. Chang, J. M. Ramseur, and M. W. Myers.** 1975. Interferon-directed inhibition of chronic murine leukemia virus production in cell cultures: lack of effect on intracellular viral markers. J. Virol. 16:569–574.
14. **Friedman, R. M., and J. M. Ramseur.** 1974. Inhibition of murine leukemia virus production in chronically infected AKR cells. A novel effect of interferon. Proc. Natl. Acad. Sci. U.S.A. 71:3542–3544.
15. **Gifford, G. E., M. V. Mussett, and E. Heller.** 1964. Comparative studies on the production and assay of interferon. J. Gen. Microbiol. 34:475–481.
16. **Gifford, G. E., and S. T. Toy.** 1970. Assay of interferon. General comments, p. 80–91. *In* F. T. Perkins and R. H. Regamey (ed.), International Symposium on Interferon and Interferon Inducers. S. Karger, Basel.
17. **Hermodson, S., and L. Phillipson.** 1963. A sensitive method for interferon assay. Proc. Soc. Exp. Biol. Med. 114:574–579.
18. **Knight, E., Jr.** 1975. Heterogeneity of purified mouse interferon. J. Biol. Chem. 250:4139–4144.
19. **Liberman, D., Z. Voloch, H. Aviv, U. Nudel, and M.**

Revel. 1974. Effects of interferon on hemoglobin synthesis and leukemia virus production in Friend cells. Mol. Biol. Rep. 1:447-451.
20. Lindemann, J., and G. E. Gifford. 1963. Studies on vaccinia virus plaque formation and its inhibition by interferon. III. A simplified plaque inhibition assay of interferon. Virology 19:302-309.
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
22. McAllister, R. M., M. Nicholson, M. B. Gardner, R. W. Rongey, S. Rasheed, P. S. Samra, R. J. Huebner, M. Hatanaka, S. Oroszlan, R. V. Gilden, A. Kabigting, and L. Vernon. 1972. C-type virus released from cultured human rhabdomyosarcoma cells. Nature (London) New Biol. 235:3-6.
23. McCormick, J. J., L. J. Larson, and M. A. Rich. 1974. RNase inhibition of reverse transcriptase activity in human milk. Nature (London) 251:737-740.
24. McWilliams, M., M. S. Finkelstein, P. T. Allen, and D. J. Giron. 1971. Assay of chick interferons by inhibition of viral ribonucleic acid synthesis. Appl. Microbiol. 21:959-961.
25. Pidot, A. L. R. 1971. Dye uptake assay: an efficient and sensitive method for human interferon titration. Appl. Microbiol. 22:671-677.
26. Salzberg, S., M. S. Robin, and M. Green. 1973. Appearance of virus-specific RNA, virus particles and cell surface changes in cells rapidly transformed by the murine sarcoma virus. Virology 53:186-195.
27. Sedmak, J. J., and S. E. Grossberg. 1973. Interferon bioassay: reduction in yield of myxovirus neuraminidase. J. Gen. Virol. 21:1-7.
28. Sedmak, J. J., S. E. Grossberg, and R. Jameson. 1975. The neuraminidase yield-reduction bioassay of human and other interferons. Proc. Soc. Exp. Biol. Med. 149:433-438.
29. Sonnabend, J. A., and R. M. Friedman. 1973. Mechanisms of interferon action, p. 201-239. *In* N. B. Finter (ed.), Interferons and interferon inducers. North Holland Publishing Co., Amsterdam.
30. Stewart, W. E., II, E. De Clercq, and P. De Somer. 1972. Cellular alteration by interferon: a virus-free system for assaying interferon. J. Virol. 10:896-901.
31. Temin, H. M., and D. Baltimore. 1972. RNA directed DNA synthesis and RNA tumor viruses. Adv. Virus Res. 17:129-186.
32. Tilles, J. G., and M. Findland. 1968. Microassay for human and chick cell interferons. Appl. Microbiol. 16:1706-1707.
33. Vilcek, J., M. H. Ng, and T. G. Rossman. 1968. Studies on the action of interferon in cell-free systems, p. 185-196. *In* G. Rita (ed.), The interferons. Academic Press Inc., New York.