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Relative Sensitivities of Viruses to Different Species of Interferon

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Some viruses were found to be more sensitive than others to the action of interferons from certain species of animals but less sensitive to interferons from other species. Vaccinia virus was the most sensitive to mouse and hamster interferons of five viruses tested, but the least sensitive of these five viruses to human, rabbit, and bat interferons. The relative sensitivities of the viruses to interferons were found to be characteristic for each of the species tested, with those closely related phylogenetically exhibiting similar patterns of relative interferon-induced virus resistance. The amount of synthetic double-stranded polynucleotide polyinosinic acid-polycytidylic acid required to induce resistance to each of the viruses in each of the cell species correlated with the interferon sensitivities of the viruses.

Interferons are characterized by their ability to induce cells to become resistant to a wide spectrum of unrelated viruses. However, this virus, resistant state is not uniform, since cells treated with a given concentration of interferon will become extremely resistant to some viruses and less resistant to others; thus, viruses have been referred to as interferon-sensitive or interferon-insensitive without referring to the species of interferon involved (14). From reports in the literature, it appears that certain viruses are relatively sensitive to some interferons but relatively insensitive to interferons from other species. Several investigators (1, 11, 18, 20) have found vaccinia virus to be more sensitive than vesicular stomatitis virus (VSV) to chick and mouse interferons, but others (9) found the reverse sensitivities to human interferon. Similar situations have been found with the sensitivity of vaccinia and Sindbis viruses to rat interferon (6) and human interferon (13), and with the sensitivity of vaccinia and Semliki Forest viruses (SFV) to chick interferon (18) and bovine interferon (8).

Since the mechanism by which interferons influence viral biosynthesis is thought to be the same for all viruses, it has been proposed that differences in sensitivities of viruses to interferon result from a sensitive virus containing, in its polycistronic message, more points that are sensitive to the action of the proposed translation-inhibitory protein(s) (TIP) of the interferon system (15, 16). Therefore, the finding that viruses have different relative sensitivities to different species of interferons would not seem to be compatible with the proposed TIP mechanism of action of interferons.

It seemed possible that the apparent discrepancies in reports on the sensitivities of viruses to the same interferon species (2), as well as the differences in relative sensitivities of viruses to different interferons, could be attributed to variations in systems in individual laboratories or to virus strain differences. The present study was designed to determine whether a selected group of viruses differed in their relative sensitivities to interferons from a variety of host species and to establish whether relative sensitivities to the resistance induced by an interferon is a characteristic of the host species.

MATERIALS AND METHODS

Animals. Pregnant albino Swiss mice, obtained from the Euers' Farm, Austin, Tex., were used as the source of mouse embryos. Weanling golden hamsters were obtained from the departmental hamster colony. Infant albino rabbits, 10 to 12 days old, were provided by R. A. Finkelstein of this department. Pregnant Mexican free-tailed bats (*Tadarida brasiliensis*), netted in caves in south Texas, were used as a source of bat embryos.

Viruses. Vaccinia virus, strain CL, obtained from the American Type Culture Collection (ATCC), was inoculated onto BHK-21 monolayer cultures, and a stock virus suspension was prepared from culture fluids collected 24 to 48 hr postinoculation. Sindbis viruses, small and large plaque variants, designated SBS and SBL, respectively, were obtained from B. P. Sagik of The University of Texas at Austin. Stock suspensions of these viruses were prepared in the same manner as described for vaccinia virus. SFV, the original strain from ATCC, and VSV, obtained from Robert Hanson of The University of Wisconsin at Madison, were also prepared in this manner, as was Japanese B encephalitis (JBE) virus, strain OCT-541,

which was supplied by W. M. Hammon of The University of Pittsburgh. A stock of St. Louis encephalitis (SLE) virus, strain CC-16, obtained originally from the blood of a naturally infected Mexican free-tailed bat by R. R. Allen of this department, was prepared from the sixth suckling mouse brain passage by inoculation onto BHK-21 cell cultures. A stock of Newcastle disease virus (NDV), California strain, obtained from ATCC, was prepared from the 25th passage in 9- to 11-day-old chick embryos.

Tissue cultures. Primary hamster kidney (HK) monolayer cultures were prepared by inoculating plastic petri dishes (60 × 15 mm) with approximately 3×10^7 cells in 3 ml of medium 199 with 10% calf serum and antibiotics (penicillin and streptomycin). Plates were incubated at 37 C in 5% CO_2 for 48 hr, at which time growth medium was replaced by 3 ml of fresh medium. Monolayers were complete by the 5th day.

Bat embryo tissue cultures, designated TBE, were prepared from embryos 1.5 to 2.0 cm in length. Cultures were prepared by inoculating 60-mm plates with 1.5×10^6 cells in 3 ml of Eagle minimal essential medium (MEM) with 10% fetal calf serum and antibiotics. Monolayers were complete after 24 hr. Two clones prepared from these cells at the 10th passage were used in these studies. These clones, designated TBE-31 and TBE-42, were equally sensitive to interferon, but only TBE-31 produced interferon. The preparation and properties of these clones will be described elsewhere (Stewart and Sulkin, *in preparation*). All TBE cells used in these studies were between 15 and 35 passages.

Rabbit kidney (RK) monolayer cultures were prepared by inoculating 60-mm plates with 2×10^6 cells in 3 ml of MEM with 10% calf serum and antibiotics. These cultures were ready for use after 72 hr of incubation.

Mouse embryo monolayer cultures were prepared by inoculating 60-mm plates with 2×10^6 cells in 3 ml of MEM with 10% calf serum and antibiotics and incubating for 48 hr.

Human embryonic lung (HEL) cells were obtained from Flow Laboratories, Rockville, Md. Monolayer cultures were prepared by inoculating 60-mm plates with 10^6 cells in 3 ml of MEM with 10% calf serum and antibiotics and incubating for 72 hr.

Preparation of synthetic double-stranded polynucleotide. Polyinosinic acid (poly I) and polycytidylic acid (poly C) were obtained from Mann Laboratories, New York, N.Y.; both were stored at −20 C at a concentration of 250 μg/ml in phosphate-buffered saline (PBS) at *p*H 7.0 until used to prepare double-stranded complex (poly IC). The optimal ratio for complexing of poly I and poly C was determined by mixing various proportions of each compound at a concentration of 25 μg/ml in PBS for 30 min at 37 C. The time required for maximal complex formation was determined by measuring the hypochromic effect at 260 nm in a Beckman model DU spectrophotometer at intervals during incubation of the compounds. The hypochromic effect was greatest when equal molar concentrations of the nucleotides were incubated for 2 hr. In studies involving poly IC, the complex was prepared by mixing equal molar concentrations of poly I and poly C for 2 hr at 37 C.

Interferon production. Hamster interferon was prepared by inoculating weanling hamsters with 100 μg of poly IC intraperitoneally. The animals were bled from the heart 6 hr after injection, and the serum obtained was dialyzed at 4 C against *p*H 2.0 Hanks balanced salt solution for 24 hr and then redialyzed to *p*H 7.0. The serum was then centrifuged at 105,000 × *g* for 2 hr, and the top three-fourths of the supernatant fluid was stored at 4 C.

Bat interferon was prepared by inoculating TBE-31 monolayer cultures in plates (100 × 20 mm) with 5 ml of growth medium containing 25 μg of poly IC per ml. After incubating the plates for 1 hr at 37 C, the medium was replaced with 20 ml of growth medium, and cultures were reincubated for 18 hr. The medium was then collected and processed for interferon in the same manner as described for hamster serum.

Mouse, rabbit, and human interferons were prepared by inoculating monolayer cultures in 100-mm plates with NDV at a multiplicity of approximately 1 plaque-forming unit (PFU) per cell. After adsorption for 1 hr at 37 C, inocula were replaced with 20 ml of growth medium. The cultures were incubated for 18 hr, at which time media were removed and processed for interferon as previously described.

Characterization of interferons. The interferon preparations were characterized as resistant to *p*H 2.0 and nonsedimentable at 105,000 × *g*. Each interferon was tested for activity against at least five different viruses in homologous cells and for cross-reactivity in the other four cell systems, with the most sensitive virus in each cell system. Interferons were tested for trypsin sensitivity and for direct activity against virus.

Interferon assays. Interferons were assayed by a plaque reduction method similar to that described by Wagner (21). Monolayer cultures of TBE-31, TBE-42, RK, HK, HEL, and ME cells in 60-mm plates were incubated with 2 ml of twofold dilutions of the appropriate interferon, with three plates per dilution. After 18 hr of incubation, interferons were aspirated, monolayers were washed twice with growth medium, and 0.5 ml of virus suspension was added. Approximately 100 PFU of vaccinia virus, 50 PFU of VSV, SBS, SBL, and SFV, and 75 PFU of SLE and JBE viruses were added to interferon-treated and control cultures. After adsorption at 37 C for 1 hr, inocula were removed and 3 ml of overlay medium, consisting of 0.5% lactalbumin hydrolysate, 2% fetal calf serum, 1% glutamine, antibiotics, and 0.5% agarose adjusted to *p*H 7.2 to 7.4 with $NaHCO_3$, was added to each plate. These plates were incubated at 37 C in 5% CO_2 until plaques developed. Overlay medium was then removed, cell sheets were stained with 0.2% aqueous crystal violet, and plaques were counted. All viruses were assayed simultaneously by using the same passage of cells and the same preparation of each interferon. Each species of interferon was assayed on three separate occasions against all viruses. Results were recorded as per cent of control plaque counts. Plaque reduction between 20 and 80% was plotted, and the 50% plaque-depressing dose (PDD_{50}) end

point for each interferon against each virus was determined.

Poly IC assays. A plaque reduction method similar to that described for interferon assays was used to determine the amount of poly IC needed to give 50% plaque reduction of each of the viruses in the various host systems. Monolayer cultures of each of the cell types in 60-mm plates were incubated for 24 hr with 2 ml of growth medium containing poly IC at concentrations of 20, 10, 5, 2.5, 2.0, 1.0, 0.5, 0.25, 0.1, 0.05, and 0.025 μg/ml. Cultures were then washed and assayed for resistance to each of the viruses. All viruses were assayed simultaneously in each cell type, and each assay was performed three times with freshly prepared poly IC each time. The PDD_{50} of poly IC for each virus was determined in each of the systems by the same procedure used for determining the PDD_{50} of interferon.

RESULTS

Development of plaques in the cell systems. Before determining the relative sensitivities of the viruses to the interferons, the plaque-forming ability of each of the viruses in each cell system was studied (Table 1). The only cells to produce plaques with SLE virus were HK, and JBE virus produced plaques in only HK and TBE-42 cells.

Characterization of the interferons. Each interferon preparation was characterized as nonsedimentable at 105,000 × *g*, nondialyzable, not inactivated at *p*H 2.0, and virus-nonspecific. None of the interferons was directly antiviral when incubated with VSV, and none showed any cytotoxic effect when incubated on cells for 18 hr. All were inactivated by trypsin. Strict species specificity was not observed with all interferons. Mouse interferon was partially active in hamster cells, producing more than 1% of its homologous titer, and hamster interferon was active in mouse cells to about 6% of its homologous titer (Table 2). All other interferons were species-specific.

Relative sensitivities of viruses to interferons. To determine the sensitivities of the viruses to each of the interferons, plaque reduction assays were performed simultaneously against the viruses found to produce plaques in the cell system involved. The average per cent reduction of plaques in three separate determinations was plotted for each dilution of interferon, and the 50% end point for each virus was determined graphically (Fig. 1).

Vaccinia virus was the most sensitive of the five viruses to mouse interferon. SFV was 32 times less sensitive than vaccinia virus, and 15 times less sensitive than VSV.

Hamster interferon was assayed against seven viruses. SFV was again the least sensitive, with less than 20% plaque reduction with undiluted interferon. Vaccinia virus was also the most sensitive of the group to hamster interferon, being 61 times more sensitive than SLE virus. VSV was again of moderate to high sensitivity, and SBS, SBL, and JBE were relatively insensitive.

The relative sensitivities to human interferon showed an interesting contrast to those found with mouse and hamster interferons. Both Sindbis viruses were more sensitive than the other three viruses. Vaccinia virus, which was the most sensitive to hamster and mouse interferons, was the least sensitive to human interferon. VSV, as before, was fairly sensitive, and SFV was again relatively insensitive.

Bat interferon presented another order of sensitivities. In TBE-31 cells, only five viruses which would form plaques were tested. VSV, the most sensitive of these five, was 15 times more sensitive than vaccinia virus, which was the least sensitive. SFV was fairly sensitive to this interferon, and both Sindbis viruses were less sensitive. In TBE-42 cells, which produced plaques with JBE virus, the same order of sensitivities of these

TABLE 1. *Development of plaques in cell culture systems*

Virus	Cell culture system											
	Hamster kidney		Rabbit kidney		Mouse embryo		Human embryonic lung		Bat embryo (clone 31)		Bat embryo (clone 42)	
	Size[a]	Time[b]	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
Vaccinia	1	72	2	72	2	72	2	72	1	72	2	72
Vesicular stomatitis	4	48	2	48	3	48	3	48	2	48	2	48
Semliki Forest	5	48	2	72	5	48	4	48	3	72	4	48
Sindbis (small)	3	48	3	96	4	72	3	72	4	96	3	96
Sindbis (large)	3	48	4	96	4	72	4	96	3	96	3	96
Japanese B encephalitis	2	96	—[c]	—	—	—	—	—	—	—	2	96
St. Louis encephalitis	2	96	—	—	—	—	—	—	—	—	—	—

[a] Diameter of plaques in millimeters.
[b] Hours required for plaques to develop.
[c] No plaques developed.

five viruses was observed, but JBE virus was approximately 10 times more sensitive than VSV and 100 times more sensitive than vaccinia virus.

Rabbit interferon was very active against VSV, SFV, SBS, and SBL viruses, but vaccinia virus was about 250 times less sensitive than any of these viruses.

TABLE 2. *Cross-reactivities of interferons from different host species*

Species of interferon	Titers in various cell cultures				
	Mouse embryo	Human embryonic lung	Hamster kidney	Bat embryo (clone 31)	Rabbit kidney
Mouse	2,250[a]	—[b]	32	—	—
Human	—	230	—	—	—
Hamster	4	—	60	—	—
Bat	—	—	—	38	—
Rabbit	—	—	—	—	6,140

[a] Homologous titers as determined by titration of interferons with challenge virus most sensitive to interferon tested.

[b] Indicates titer of less than 4 PDD_{50} units against virus most sensitive to interferon tested.

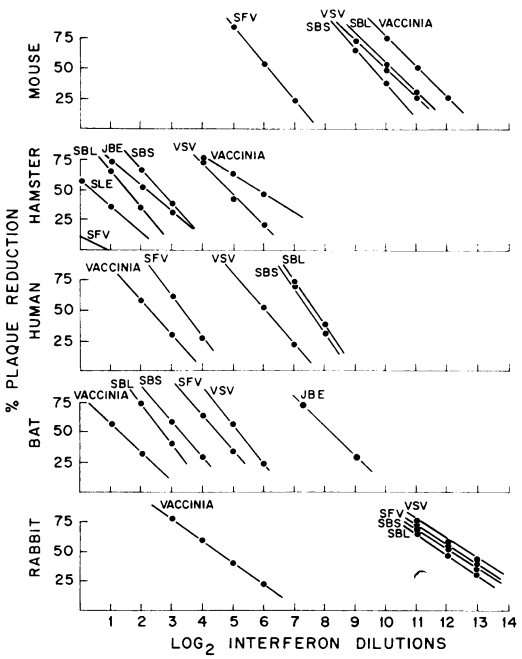

FIG. 1. *Relative sensitivities of viruses to interferons. Each point represents the average plaque reduction obtained in three separate assays. Mouse interferon was assayed in mouse embryo cells; hamster interferon in hamster kidney cells, human interferon in human embryonic lung cells; and bat interferon in bat embryo cells, clone 31 and clone 42. JBE virus was assayed only in clone 42 cells.*

Relative sensitivities of viruses to poly IC-induced resistance. It could be argued that the interferon preparations, having come from in vivo and in vitro sources, contain materials other than interferons which could contribute in some way to the differences in relative sensitivities observed. To investigate this possibility, all cell types were treated with several concentrations of poly IC and then challenged with each of the viruses used in the interferon assays. Since poly IC acts by inducing the interferon system, each of the viruses should be inhibited by that amount of poly IC necessary to induce the interferon response sufficient to inhibit each of the challenge viruses, so that the amount of poly IC required to inhibit each virus should be greater for those viruses resistant to that species of interferon, and less for the viruses sensitive to that interferon. The viruses showed a wide variation in susceptibilities to poly IC-induced resistance (Table 3). The relative poly IC sensitivities correlated with the relative interferon sensitivities in ME, RK, HEL, and TBE-31 cells. The different cell systems also varied in their responses to poly IC. TBE-31 and RK cells seemed to be very sensitive, and HEL and ME cells were slightly less sensitive. In HK cells, no inhibitory activity was detected against any of the challenge viruses, even at a concentration of 20 μg of poly IC per ml. Also, TBE-42 cells, which do not seem to produce interferon but are sensitive to it, were not protected against any of the challenge viruses by treatment with 10 μg of poly IC per ml. These

TABLE 3. *Relative sensitivities of viruses to poly IC-induced resistance*

Virus	PDD_{50} of poly IC in indicated cells[a]			
	Mouse embryo	Human embryonic lung	Bat embryo (clone 31)	Rabbit kidney
Vaccinia	0.5	20	5.0	10
Semliki Forest	20	10	0.1	0.025
Sindbis (small)	2.5	1.0	0.5	0.025
Sindbis (large)	1.0	1.0	1.0	0.025
Vesicular stomatitis	1.0	2.5	0.05	0.025
Japanese B encephalitis	ND	ND	ND	ND
St. Louis encephalitis	ND	ND	ND	ND

[a] Concentration of poly IC (μg/ml) required to reduce plaques to 50% of control counts; cultures incubated with 2 ml of poly IC in growth medium. ND indicates assays not done. With hamster kidney and bat embryo (clone 42) cells, there was no reduction with 20 μg of poly IC per ml.

experiments indicate that the results obtained with the crude interferon preparations were not due to impurities, but that viruses have different relative sensitivities to different species of interferon. It was also found that poly IC can be used to determine the relative sensitivities of viruses to an interferon, if the cells used in the assay are responsive to poly IC.

Induction of interferon in the cell systems. The induction of interferon by the viruses during the assay for interferon sensitivities could contribute to the relative inhibition of viruses in each system. To investigate the contribution of induced interferon to the relative inhibition of viruses, experiments were performed to correlate the amount of interferon induced by the viruses with their relative sensitivities to the interferon induced. Monolayer cultures of TBE-31 and TBE-42, each containing approximately 3.5×10^6 cells, were exposed to VSV, SFV, SBS, SBL, or vaccinia viruses at a multiplicity of approximately 1 PFU per cell. After incubating the cultures at 37 C for 18 hr, media were collected, processed, and assayed for interferon against VSV on TBE-31 cells. None of these viruses induced detectable interferon in TBE-42 cultures, even when media were concentrated more than 10-fold by dialysis against Carbowax. Media from TBE-31 cultures exposed to SFV or SBS contained 80 units of interferon per ml, those exposed to SBL contained 40 units per ml, and those exposed to VSV or vaccinia virus contained 20 units per ml. As shown above, TBE-42 and TBE-31 were sensitive to interferon, and both gave the same orders of relative sensitivities of the viruses to interferon. This suggests that induction of interferon in the assay systems is not a significant factor influencing the results. In other experiments, HK cells similarly exposed to vaccinia virus, which is sensitive to hamster interferon, produced no detectable interferon, whereas SFV, which is very insensitive to hamster interferon, induced 40 units per ml in these cultures. It is concluded, therefore, that induction of interferon during assays is probably not responsible for the differences in relative sensitivities obtained.

DISCUSSION

These experiments show that each species of interferon has a characteristic spectrum of activity against viruses. Although virus "A" may be much more sensitive than virus "B" to one species of interferon, the reverse situation may be found with another interferon species. Vaccinia virus was the most sensitive of the five viruses tested to hamster and mouse interferons, but was the least sensitive of these same viruses to human, bat, and rabbit interferons, whereas SFV, the least sensitive of the five viruses to hamster and mouse interferons, was relatively sensitive to bat and rabbit interferons. Therefore, when a virus is referred to as interferon-sensitive or interferon-insensitive, the species of interferon involved must be stated.

Marcus and Salb (15) proposed that interferons act against all viruses by the same mechanism, i.e., by inducing a TIP which can selectively inhibit readout of messenger ribonucleic acids. The more sensitive a virus is to an interferon, the more sites it must have on its polycistronic message that are sensitive to the action of the TIP (16). However, if all species of TIP acted on the same sites, all species of interferon would inhibit viruses in the same order; i.e., virus "A" would be more sensitive than virus "B" to all species of interferons. Since this was not found to be the case in the present studies, it seems that the concept of TIP may need further investigation.

The relative sensitivities obtained in these studies are in agreement with all reports in the literature, suggesting that the previously reported differences are not entirely due to variations in virus strains or to systems employed in individual laboratories. The relative sensitivities of vaccinia virus and VSV to mouse interferon agree with those reported by Glasgow and Habel (11). The results with rabbit interferon show both VSV and Sindbis virus to be sensitive, as reported by Ho (12). Sindbis virus was found to be more sensitive than vaccinia virus to human interferon, corresponding to the results of Ho and Enders (13). Gallager and Khoobyarian (9) found VSV to be more sensitive than vaccinia virus to human interferon, which was the same relationship found in these studies.

The cross-reactivity of mouse and hamster interferons agrees with the report by Buckler and Baron (3), and the similarities of the orders of sensitivities of viruses to hamster and mouse interferons and those reported for rat interferon by De Maeyer and De Somer (6) suggest a phylogenetic relationship in patterns of interferon sensitivities. Studies currently being carried out to determine the relative sensitivities of viruses to monkey interferon and to another genus of bat interferon should clarify this point. The lack of cross-reactivity of human interferon in rabbit cells is in contrast to the reports of Desmyter et al. (7), Merigan (*personal communication*, 1969), and Levy, Golgher, and Paucker (Bacteriol. Proc., 1969, p. 169), who showed human interferon to be active in rabbit cells. However, the human interferon used in our study was a crude preparation of relatively low potency and was from a different

cell type. Where we have, for convenience, referred to the sensitivities of viruses to interferons, we actually should refer to the resistance induced in cells by these inhibitors. In this regard, it is particularly interesting that the cell species with interferons that cross-react, i.e., mouse and hamster cells, exhibited similar relative orders of interferon-induced resistance to the viruses, as did human and rabbit cells, which have been reported to show a one-way cross reactivity (7).

The correlation between poly IC-induced resistance and interferon-induced resistance shows that the relative responses of viruses to poly IC may be used to determine the relative interferon sensitivities of viruses. This would be particularly helpful when one is initiating studies involving the interferon system in an animal species that has not previously been shown to produce interferon and for which neither a standard interferon is available nor the appropriate challenge virus known.

The fact that HK cells were not responsive to poly IC can be explained in three ways: the cells are deficient in producing interferon, they fail to respond to interferon, or they fail to take up poly IC. The assay system for hamster interferon shows that HK cells are sensitive to interferon and produce interferon when exposed to SFV; HK cells have also been shown to produce interferon when infected with other viruses (5). Therefore, it seems that lack of uptake of poly IC by HK cells is the reason for their unresponsiveness to it. Billiau and associates (*personal communication*) found that African green monkey kidney cells and chick embryo cells are unresponsive to poly IC unless polybasic compounds such as neomycin and streptomycin are added; these compounds presumably aid in cellular uptake of the interferon inducer. The finding that poly IC did not protect TBE-42 cells, which do not produce interferon but are sensitive to it, suggests that, if this compound is taken up by these cells, poly IC acts only on the cistron for interferon production and not on the cistron for production of the inhibitory proteins of the interferon system. This is supported by the findings of Schafer and Lockart (Bacteriol. Proc., p. 149, 1969).

The studies summarized in this report show that the relative sensitivities of viruses to a particular species of interferon must be determined by assaying them against that species, not by inference from assays against another interferon species. Rokutanda (19) states that two strains of JBE virus which differ in virulence for mice have the same sensitivities to interferon, but she determined their sensitivities to chick interferon, not mouse interferon. It is apparent from the results presented in Fig. 1 that two viruses can have the same sensitivities to one species of interferon (VSV and SFV with rabbit interferon) but quite different sensitivities to another species of interferon (VSV and SFV with hamster interferon). Similarly, Wagner et al. (22) found that two variants of VSV which show the same sensitivities to chick interferon have different sensitivities to mouse interferon.

Production of interferon during the assays was apparently not a significant factor influencing the results. Gifford (10) drew a similar conclusion when he found that vaccinia virus, which is very sensitive to chick interferon, induces very little interferon in chick cells. The size of the plaques produced did not present any clear correlation with sensitivities to the interferons. Vaccinia virus and both Sindbis viruses produced plaques of about the same diameter in all of the cells, regardless of their sensitivities to the particular interferon involved. VSV also produced the same size plaques in each of the cells but was relatively sensitive to all of the interferons. It is interesting that the only cells that produced plaques with JBE virus were HK and TBE-42. JBE virus is very sensitive to bat interferon, but TBE-42 cells do not produce interferon. The arboviruses were generally more resistant to hamster interferon than they were to the other interferons, and this could paritally explain their greater ability to produce plaques in HK cells.

Several investigators have reported that adenoviruses are very insensitive to interferon (4, 9, 18). It is tempting to speculate that the adenoviruses, if assayed against the right species of interferon, may be more interferon-sensitive than viruses that are currently considered to be interferon-sensitive.

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