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Cellular Resistance to Induction of Interferon[1]

KURT PAUCKER AND MARTHA BOXACA

The Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19146

INTRODUCTION

The literature contains numerous reports which indicate that the response of host cells to viral stimulation of interferon production can be modified in various ways. Thus, it was shown that fragments of chick chorioallantoic membrane, in which interferon had been induced by heated influenza virus, could be restimulated a second time not only by inactivated virus (5, 23), but also by live influenza virus which normally did not elicit interferon in this system during the time interval tested (5, 13). A comparable enhancement effect was noticed when pieces of chorioallantoic membrane were first treated with adequate doses of interferon and subsequently infected with live influenza virus (13, 15), resulting in the early appearance of interferon in the medium. Pretreatment of L cells with partially protective doses of interferon gave rise to higher titers of interferon on infection with Western equine encephalitis virus than in untreated controls (24). Others have reported recently that priming of chick embryo fibroblasts with low doses of interferon evoked a more rapid and enhanced production of interferon in response to infection with Chicungunya virus (8*b*, 21*a*).

In contradistinction to these examples of increased cellular activity caused by a preconditioning contact with interfering viruses or with interferon itself, other observations disclosed quite the opposite phenomenon under similar circumstances. For example, the completion of interferon synthesis in L cells (6) and in chick embryo fibroblasts (4) has been associated with a state of resistance to further stimulation of interferon production. Similarly, pretreatment with interferon was found to preclude initiation of interferon formation in chick embryo fibroblasts by tick-borne encephalitis virus (34, 35) and Chicungunya virus (8*b*), and in L cells by Newcastle disease virus (6) as well as by Western equine encephalitis virus (24).

These seemingly contradictory findings point to the fact that the response of cells to stimulation of interferon production and to the action of exogenous interferon may be subject to considerable variation. In the last analysis, cellular behavior may be dictated largely by the quantitative and temporal considerations governing the experimental procedures employed. The present report describes some recent observations on the establishment and persistence of nonresponsiveness to viral induction of interferon in L-cell cultures.

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RESISTANCE TO RESTIMULATION OF INTERFERON PRODUCTION IN L CELLS

Newcastle Disease Virus-induced Synthesis of Interferon

A series of earlier publications (6, 7, 31–33) established the suitability of suspended cultures of L cells, Newcastle disease virus (NDV), and vesicular stomatitis virus (VSV) for quantitative studies on viral interference, interferon synthesis, and interferon action. These references may be consulted for all pertinent technical details.

It had been reported previously that the synthesis of interferon induced by irradiated Newcastle disease virus (NDV_{uv}) in L cells was largely completed within 24 hr of incubation. At that time, the cultures could not be restimulated by NDV to yield a second crop of interferon (6). The question arose, therefore, whether these two events, i.e., cessation of interferon production and resistance to its induction, occurred simultaneously, or whether they developed in the cultures at different times. However, the rapid accumulation of interferon in the cellular growth medium did not permit accurate determination of the rate at which interferon production eventually ceased, and it tended to obscure the response of the cells to secondary viral stimulation. This situation was obviated by removal of accumulated interferon from the medium at different times during the production cycle and measurement of the yields of interferon released from the freshly resuspended cells during ensuing, short time intervals (differential titers). In this way, the synthesis of interferon was followed in cell cultures which had been exposed either to viable or ultraviolet-irradiated NDV (Fig. 1 and 2). In the first instance, interferon became detectable after 4 to 6 hr of incubation and attained a peak during the 12- to 14-hr interval. Thereafter, the synthesis of interferon was abruptly shut down except for small increments which continued to be released from the cells for at least 32 hr. The perpetuation of low levels of interferon production for prolonged periods of time can probably be attributed to the stabilization of the virus in the culture and the establishment of a persistent infection (10).

Essentially similar findings were obtained with NDV_{uv} (Fig. 2), except that the rise and fall of interferon production was more protracted than with live virus and that maximal titers achieved were slightly lower. The frequent replenishment of medium appeared to favor somewhat increased yields of interferon, as is apparent from the calculated cumulative values shown in Fig. 2. This suggests that the accumulation of interferon in the medium tended to depress further release of interferon, although others report that cumula-

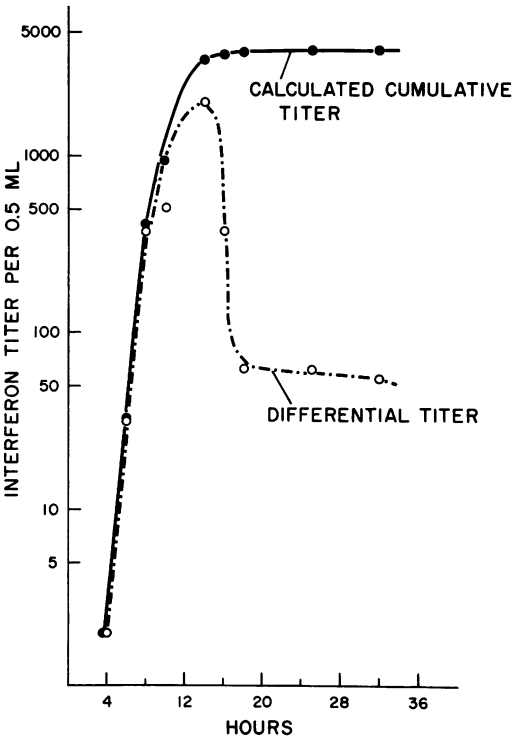

FIG. 1. *Production of interferon by viable Newcastle disease virus in L cells (29).*

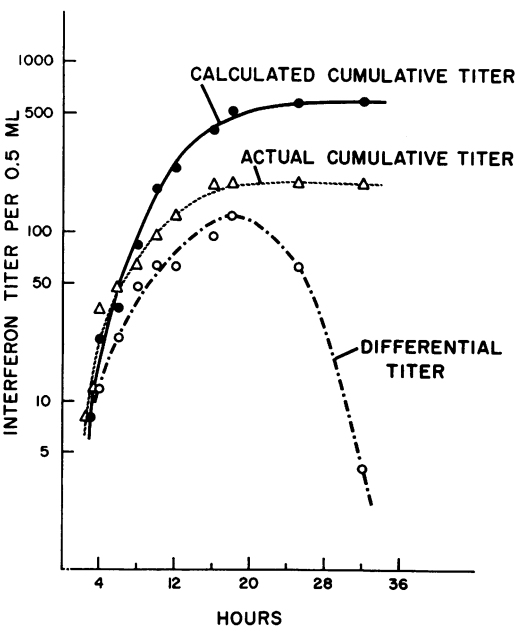

FIG. 2. *Production of interferon by ultraviolet-irradiated Newcastle disease virus in L cells (29).*

tive titers were not affected by such procedures (4).

Acquisition of Refractoriness

Determination of the rate of declining synthesis of interferon in the foregoing experiments permitted examination of the effect of a second dose of virus on interferon production already in progress. Accordingly, cells which had been subjected to a first contact with NDV_{uv} were centrifuged and resuspended in fresh medium after varying lengths of incubation, to reduce the levels of accumulated interferon to negligible proportions. At these same intervals, samples of cell suspension were exposed a second time to NDV_{uv}, and the resulting yields of interferon were compared with those which continued to be released from cultures which had not been subjected to restimulation. The data presented in Fig. 3 show that exposure of cell cultures to a second dose of NDV_{uv} at the times indicated did not enhance interferon production, and that identical levels were released from cultures stimulated once or twice. Therefore, as the synthesis of interferon first showed signs of abating, the cells exhibited already marked resistance to secondary stimulation of additional interferon formation. How much earlier refractoriness became actually established could not be determined in the presence of large amounts of interferon discharged from the cells at that time.

The establishment of protection of L cells against superinfection by VSV is gradually reinforced during the early production and release of interferon from the cells. A significant degree of antiviral resistance is attained prior to the time when the synthesis of interferon has reached its maximum. In the experiment shown in Fig. 4, suspended cultures of L cells were treated with different doses of either viable or irradiated NDV. At different time intervals thereafter, samples of cells resuspended in fresh medium were either challenged with VSV and stained 6 hr later with fluorescein-conjugated antibody to enumerate infected cells, or the accumulation of interferon during a 24-hr interval was determined with or without restimulation by NDV_{uv} as previously described. In each instance, the results were related to the number of cells infectible and yield of interferon obtainable in control cultures. Within 8 to 10 hr after first exposure to NDV_{uv}, the number of cells capable of supporting viral replication had declined considerably. On the other hand, interferon production began to fall off sharply only after 18 hr of incubation, and, as shown in Fig. 3, the cultures could not be restimulated at that time by a second dose of NDV_{uv}.

Loss of Refractoriness

The duration of the refractory state in L-cell cultures is dependent upon the conditions under which the cells are maintained. In actively dividing cultures, full responsiveness to interferon in-

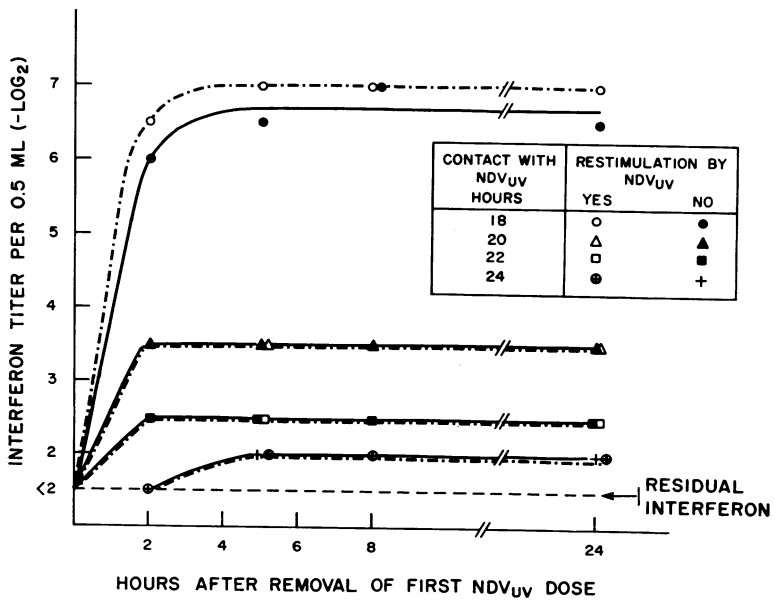

FIG. 3. *Establishment of nonresponsiveness to interferon induction by ultraviolet-irradiated Newcastle disease virus (NDV_{uv}) in L cells. Modified from Paucker and Boxaca (29).*

duction is regained rapidly, as shown in Fig. 5. In this experiment, groups of cultures were first treated for 24 hr with varying concentrations of NDV_{uv}. After substitution of fresh growth medium, the cells were permitted to grow in suspension. At different times, samples were exposed a second time to NDV_{uv} and were harvested 24 hr later to measure yields of interferon. The data show that, regardless of the size of the initial dose of NDV_{uv}, all cultures had reverted to partial responsiveness within one cell division, and, after two divisions had intervened, the cells produced as much interferon as the control cultures (6).

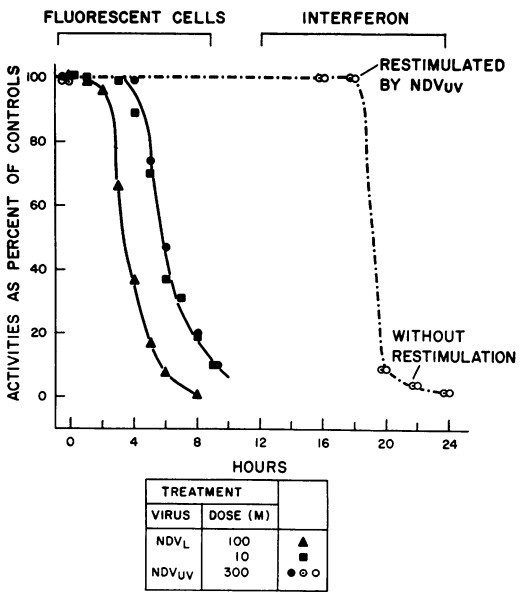

FIG. 4. *Sequential development of resistance to vesicular stomatitis virus and to viral induction of interferon in L cells treated with viable (NDV_L) and irradiated (NDV_{uv}) Newcastle disease virus (29).*

In contrast, cells which are maintained under stationary conditions retain their refractory behavior throughout the life of the culture. In the experiment illustrated in Fig. 6, L cells were exposed to NDV_{uv} (m = 100). Upon termination of the 24-hr incubation period, the resuspended cells were subdivided into two portions, one of which was densely seeded in stationary flasks in the presence of a reduced serum concentration, whereas the other was permitted to grow and divide in suspension. At intervals over an 8-day period, some cultures in both groups were inoculated a second time with NDV_{uv}, and media were harvested 24 hr later for interferon assay. No interferon could be elicited from stationary cultures during the experimental period, whereas the dividing culture had reverted from a refractory to a responsive condition. It appears, therefore,

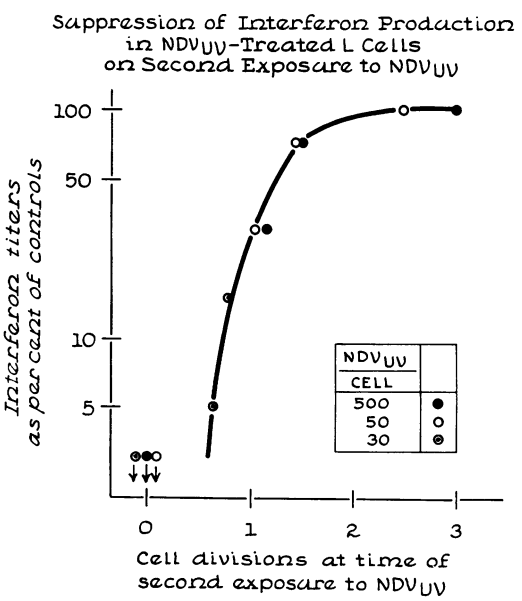

FIG. 5. *Loss of nonresponsiveness to interferon induction by irradiated Newcastle disease virus (NDV_{uv}) in L cells pretreated with varying concentrations of NDV_{uv} (6).*

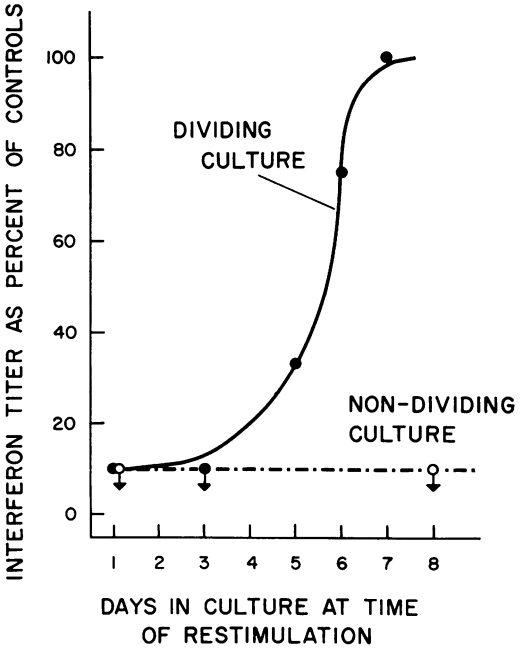

FIG. 6. *Persistence of refractory behavior in dividing and stationary cultures of L cells.*

that either the process of cell division itself or an active metabolic state is conducive to loss of the refractory property, whereas a dormant condition favors its perpetuation.

Effect of Miscellaneous Nonviral Compounds

The preceding experiments have shown that resistance to subsequent stimulation of interferon production grew out of a prior contact of the cultures with interfering virus, which resulted both in protection against superinfecting VSV and in the production of a first crop of interferon. Other compounds were therefore tested to see whether a refractory state could be initiated in the absence of either protection or interferon formation. Among the substances assayed were purified water-soluble polysaccharides extracted from dermatophytes (1) not previously associated with antiviral properties, *Escherichia coli* endotoxin, known to induce interferon only in vivo but not in tissue cultures (12), and statolon and phytohemagglutinin, both of which have been reported to elicit interferon in vitro (18, 19, 36). Groups of L-cell cultures were treated with the above compounds for 24 hr at the concentrations or dilutions indicated in Table 1. Samples were subsequently challenged with VSV to determine the degree of protection, or were exposed to NDV to measure the interferon response. With the exception of statolon, none of the compounds tested either imparted protection against VSV or diminished the interferon response to NDV. Statolon, however, exhibited a strong antiviral effect, and the cells were markedly resistant to stimulation of interferon production (29).

TABLE 1. *Viral induction of interferon in L cells pretreated with various compounds*

Pretreatment material	Dose	Results as percentage of controls	
		Infection by VSV[a]	NDV-induced interferon
	μg/ml		
Endotoxin	20	100	100
Statolon	50	<0.1	19
	16.5	<0.1	13
Mannan I	100	100	100
Mannan II	100	100	100
Glucan	100	100	100
Phytohemagglutinin	1:800[b]	100	100

[a] Determined by plaque assay or immunofluorescence.
[b] Dilution.

REFRACTORY BEHAVIOR OF L CELLS PRETREATED WITH INTERFERON

Time and Dose Relationships

The depressed response to induction of interferon is not restricted to L cells which had actively participated in interferon production prior to the time when the second stimulus was applied. This condition is also elicited in cultures pretreated with interferon preparations derived from the homologous host (6, 8*b*, 24, 35). A preincubation period with interferon for 24 hr had been routinely employed to render L cells refractory. As shown in Fig. 7, this period could not be shortened significantly without affecting the response of the cells to stimulation of interferon formation. Groups of cultures were treated for varying lengths of time with the same dose of interferon (300 plaque-inhibiting units per 2.5 × 10^7 cells). They were subsequently exposed to NDV_{uv}, and interferon titers were determined 5 and 17 hr later. The data show that the interferon response

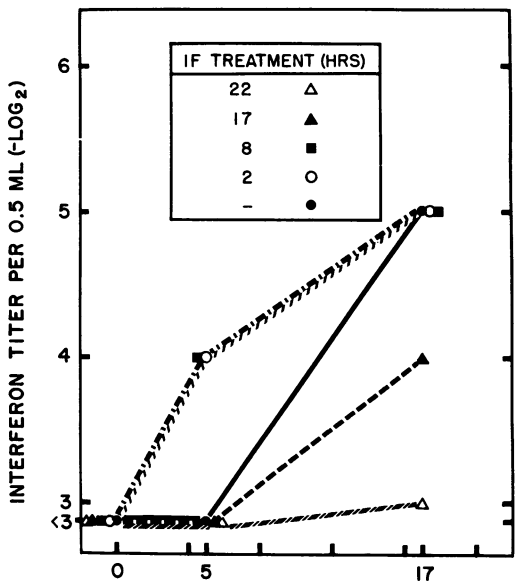

FIG. 7. *Establishment of refractoriness in L cells incubated with interferon for varying lengths of time. Dose of interferon = 300 plaque-inhibiting units per 2.5 × 10^7 cells.*

was blocked completely after treatment for 22 hr, and partially after 17 hr. Lesser times of incubation hastened the appearance of interferon in the medium (priming effect), but ultimately the same yields were produced as those obtained in controls.

The length of contact between cells and inter-

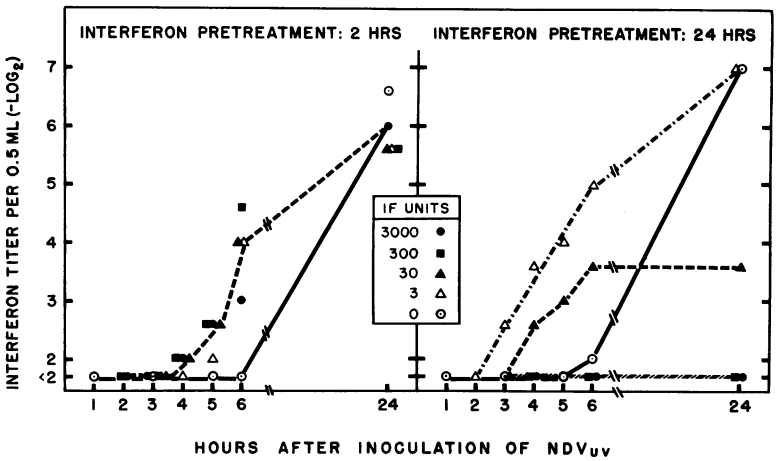

FIG. 8. *Induction of interferon in L cells pretreated with varying doses of interferon during short and long time intervals.*

feron was, however, not the only decisive factor in the development of refractoriness. The dose of interferon employed was equally important. In the experiment illustrated in Fig. 8, groups of L-cell cultures were exposed to varying concentrations of interferon for either 2 or 24 hr before being inoculated with NDV_{uv}. The appearance of interferon was followed hourly throughout the first 6 hr, and a final collection was taken at 24 hr. As expected from the experiment in Fig. 7, treatment with interferon for 2 hr, irrespective of dose, did not diminish the final yields of interferon formed. However, in all groups, measurable interferon levels preceded by at least 2 hr those of the controls, which were still negative in the 6-hr sample. Differences became apparent when pretreatment with interferon was prolonged to 24 hr. In that instance, the two largest doses containing 3,000 and 300 units, respectively, completely blocked attempts at inducing more interferon. The intermediate concentration created a partial state of responsiveness resulting in reduced yields as well as an early appearance of interferon. The lowest dose permitted the cells to respond to NDV_{uv} with full yields of interferon while, at the same time, sparking the cultures into early activity. These data point to two major differences between the priming of cells by interferon into an early response to interferon induction (15) and the elicitation of refractoriness. Priming requires neither an extended incubation with nor excessive amounts of interferon for treatment, whereas both conditions must be met to render cells refractory.

Host Specificity

In the absence of any information on the ability of heterologous interferons to induce a refractory condition in L cells, a number of such preparations derived from different species were examined with respect to this property. These materials are listed in Table 2. Treatment was for 24 hr, and the cultures were subsequently inoculated with NDV (m = 10) to measure interferon yields which were permitted to accumulate in the ensuing 24 hr. Irrespective of the method of production, human, chick, and rabbit interferons did not alter the response of the cells to stimulation of interferon formation by NDV. Only mouse interferon was capable of evoking a reduced response to viral stimulation of interferon production. Therefore, the establishment of refractoriness was

TABLE 2. *Viral induction of interferon in L cells pretreated with interferons from different species*

Interferons used for pretreatment				NDV-induced interferon	
Species	Source	Inducer	Dose[a]	Titer/0.5 ml[b]	Per cent
Human	EB1[c]	None	120	128	67
	HeLa	NDV	1,024	192	100
Chick	Egg	Influenza	200	192	100
Rabbit	RK 13	NDV	256	192	100
Mouse	L	NDV	2,000	16	8
		Sindbis	400	48	25
		None	—	192	100

[a] Expressed as 50% plaque-inhibiting units for mouse and chick interferon; 50% cytopathic effect-inhibiting units for human interferons.

[b] Determined by plaque reduction test on L-cell monolayers.

[c] Strain of lymphoblasts derived from Burkitt's lymphoma.

a property exhibited by homologous, but not by heterologous, interferon preparations.

Neutralization by Immune Serum

It is known that the antiviral effect of interferon is neutralized by prior incubation with specific immune serum prepared in guinea pigs (30) or in rabbits (28). As shown in Fig. 9, the refractoriness-inducing capacity of interferon is similarly abolished by antibody. Groups of cell cultures were first incubated for 1 hr in a small volume with either interferon alone or a mixture of interferon and anti-interferon serum in equal proportions. Controls containing only antiserum or Hanks solution were similarly incubated. Medium was then added, and the incubation was continued for the remainder of the 24-hr period. At that time, separate tubes of each group were exposed to VSV and to NDV_{uv} to determine the proportion of infectible cells and interferon response in the cultures. Both were markedly depressed by treatment with interferon, but this effect was fully neutralized in cultures pretreated with the interferon-antibody mixture.

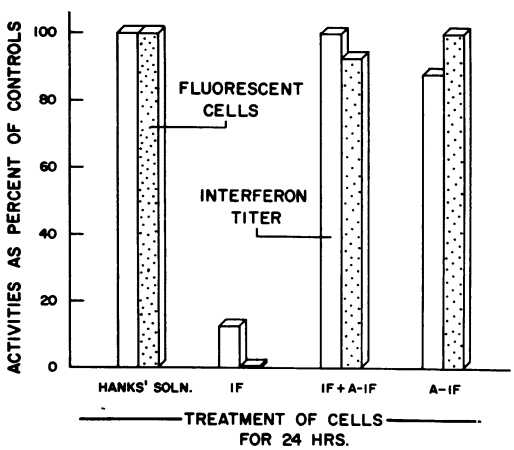

FIG. 9. *Neutralization of the refractoriness-inducing and antiviral properties of interferon by specific antibody.*

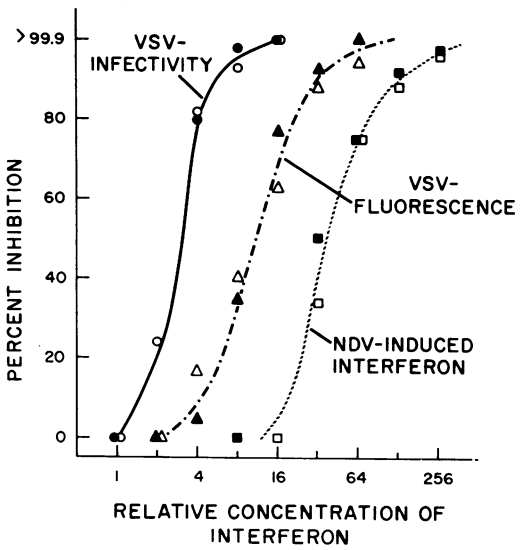

FIG. 10. *Differential effect of interferon treatment on the production of NDV-induced interferon and the suppression of vesicular stomatitis virus. Open and closed symbols represent two separate experiments.*

DISSOCIATION BETWEEN PROTECTION AND RESISTANCE TO INTERFERON INDUCTION

Quantitative Aspects

The experiments described in the preceding section suggested that the ability of interferon to reduce the cellular response to subsequent induction of interferon formation was as specific with respect to host selection and immunogenicity as its antiviral property. In this section, some observations are reported which suggest that these two expressions of interferon activity are separable and may in fact reside in different components of the crude preparations employed.

Preliminary observations disclosed that a disparity existed between the quantity of interferon required to depress the cellular response to subsequent induction of interferon and that which was needed to inhibit multiplication of a superinfecting virus to the same degree. Figure 10 depicts the depression of NDV-induced interferon yields and of VSV replication in cultures pretreated with increasing doses of interferon. Multiplication of VSV was assessed by measuring yields of virus produced in a single infectious cycle and by counting numbers of infected cells in smears stained with fluorescent antibodies. The selection of these two parameters of viral multiplication was motivated by the earlier demonstration that reliance on depression of viral infectivity alone assigns an unduly high potency to interferon preparations without taking into account large numbers of nonvirus-yielding cells capable of supporting virus growth in a limited way (25, 27, 31). It is apparent from the data presented that, to achieve a comparable degree of inhibition, from 4 to 16 times more interferon was needed to reduce interferon yields than to depress the numbers of infectible cells or infectious yields, respectively. Therefore, a selected dose of interferon may impart considerable if not total protection against infection by a virulent virus without affecting in the least the cellular response to induction of interferon by NDV.

Differential Development and Loss of Refractoriness and Protection

The finding that the levels of interferon required to inhibit viral multiplication and interferon yields were not of a similar order of magnitude suggested that these manifestations of interferon action might also be acquired by the cells at different times. The data presented in Fig. 11, which demonstrate the progressive development of resistance to VSV (as measured in terms of immunofluorescence) and to induction of interferon by NDV in interferon-treated cultures of L cells, show that this is, indeed, the case. The number of cells infectible by VSV is significantly reduced after 8 hr of incubation with interferon, whereas the interferon-producing potential of the cells is not affected until several additional hours have elapsed.

The demonstration that protection imparted to L cells by interferon is transmitted either intact or in part to descendant generations (25, 31) prompted experiments to determine whether the loss of antiviral resistance and of refractory behavior in interferon-treated cultures occurred also at different rates. The results of studies obtained in cells pretreated with two doses of interferon to establish first a solid state of protection and resistance to interferon induction are shown in Fig. 12. At regular intervals, susceptibility of the cells to VSV and to interferon induction by NDV were measured and related to the number of divisions which intervened since the initial contact with interferon. With both levels used for treatment, a partial interferon response to

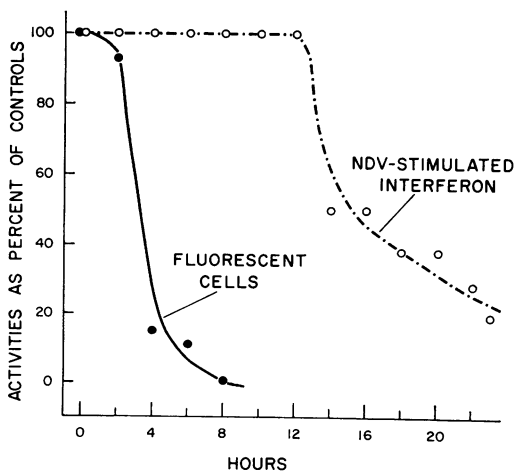

FIG. 11. *Sequential development of resistance to vesicular stomatitis virus and to induction of interferon by Newcastle disease virus (NDV) in L cells treated with homologous interferon (29).*

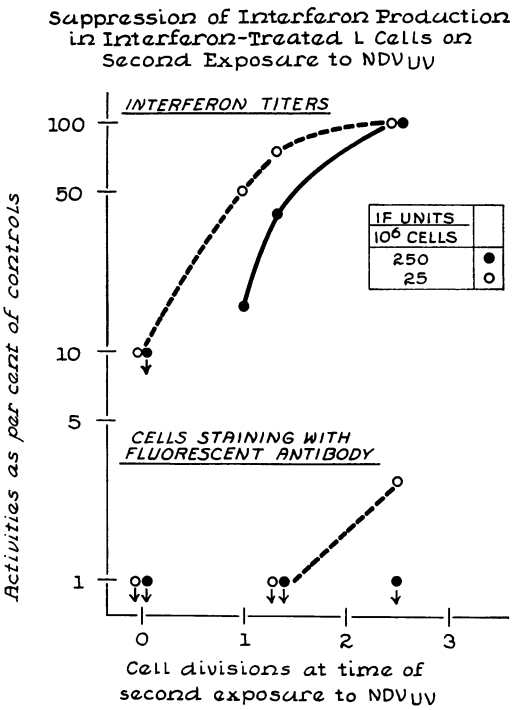

FIG. 12. *Loss of resistance to vesicular stomatitis virus and to induction of interferon by irradiated Newcastle disease virus (NDV_{uv}) in L cells treated with homologous interferon (6).*

NDV stimulation was restored already in the course of one cell division, and normal yields of interferon were elicited after two generations. In the same interval, however, the cultures remained either fully protected or, in the case of the low dose of interferon, revealed only slight susceptibility to VSV (6). Hence, a transient dissociation between these manifestations of interferon action was readily discernible in cultures in which the effect of interferon was either still increasing or already waning.

The observations recorded in Fig. 11 demonstrate that 8 hr of incubation with interferon was adequate to achieve substantial resistance of L cells to VSV, as has also been reported for Western equine encephalomyelitis virus in the same system (25). The later occurring gradual emergence of nonresponsiveness to interferon elicitation suggests two alternative explanations for this discrepancy in interferon activity. Either different aspects of interferon function can manifest themselves at different times after contact with the inhibitor, or an entirely distinct entity may be responsible for the refractory condition observed on prolonged treatment of cells with inter-

feron. To differentiate between these possibilities, the following experiment was carried out.

Cultures of L cells were exposed to interferon for different periods of time, after which the medium was replaced and incubation was continued in the absence of interferon for a total of 24 hr. At that time, the extent of protection against VSV was gauged by immunofluorescence, and levels of interferon produced by NDV were measured. These results are presented in Fig. 13. Treatment of the cultures with interferon during 2 of the 24 hr resulted in a considerable degree of resistance against VSV which was reinforced when incubation was extended to 8 hr. However, on termination of the 24-hr incubation period, these cultures, from which interferon had been prematurely removed, failed to become refractory. Only continuous incubation of the cells in the presence of interferon well beyond the time necessary for the establishment of marked if not total protection resulted ultimately in resistance to interferon induction (29). It is evident, therefore, that even though extracellular interferon was no longer present to any extent in the medium, its antiviral manifestation became increasingly pronounced as incubation of the cultures was extended, whereas under the same conditions the development of resistance to interferon formation failed to take place.

Treatment of Cells with Partially Purified Interferon

In view of the foregoing experiments, it seemed likely that a physical separation between the antiviral and refractoriness-inducing capacities of interferon preparations might be achieved. To that end, a number of materials which had been subjected to various treatments were examined for their potency in relation to antiviral titer to render cells refractory. Arbitrarily, cultures were considered refractory when the quantity of interferon produced on stimulation with viable or irradiated NDV amounted to 25% or less of that in untreated control cultures. Among the preparations tested, some had been dialyzed only against *p*H 2 (5). Others were treated with perchloric acid, followed, in some cases, by concentration with zinc acetate (20). Some materials were concentrated by ultrafiltration, subjected to dialysis against sodium acetate (33*a*), and assayed either at that stage or after elution from diethylaminoethyl-Sephadex and CM-Sephadex by a procedure to be reported elsewhere (Berman and Paucker, *to be published*). Cultures were treated with serial dilutions of these materials for 24 hr and were subsequently exposed to NDV or NDV_{uv} to assess the interferon response. In each case, the minimal dose of interferon was determined which was capable of rendering the cells refractory as defined above. The results in Table 3 show that the approximate amount of antiviral activity needed to depress the cellular response to interferon induction was not the same in all instances. In particular, those materials which, after elution from CM-Sephadex, had attained the highest degree of purity (in the order of 0.1 μg of protein per unit of interferon activity) were much less potent in blocking NDV-induced interferon than the other preparations, among which no clear-cut differences were discernible. It appears likely, therefore, that further purification will ultimately abolish the refractoriness-inducing capacity of interferon altogether. Efforts are in

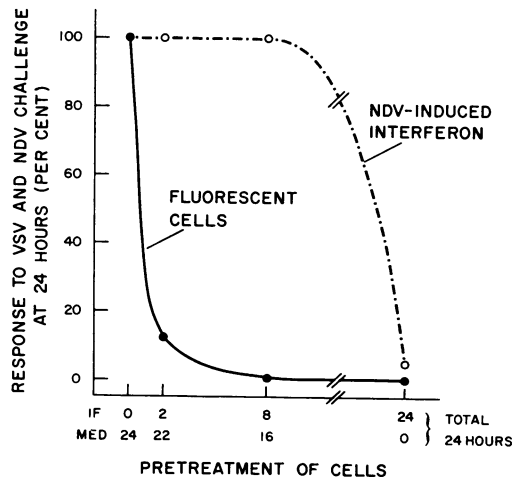

FIG. 13. *Effect of discontinuous incubation of L cells with interferon on the development of resistance to vesicular stomatitis virus (VSV) and to induction of interferon by Newcastle disease virus (NDV). Modified from Paucker and Boxaca (29).*

TABLE 3. *Refractoriness-inducing activity of interferons subjected to various treatments*

Interferon prepn		IFU/RID[a]
No.	Treatment	
87	*p*H 2	40
89	*p*H 2	16
93	*p*H 2	10
98	*p*H 2	34
89	Perchloric acid	105
93	Perchloric acid, ZnAc	40
98	Ultrafiltration, NaAc	26
93	CM-Sephadex	960
102	CM-Sephadex	>670

[a] Minimal number of interferon units per refractoriness-inducing dose (see text).

progress to isolate and characterize the as yet elusive factor responsible for this effect. It has so far not been detected in any of the major interferon-free protein peaks collected in the course of chromatographic separation.

DISCUSSION AND CONCLUSIONS

A number of systems have been described in which multiplication of a virus causes the cells to be resistant to viral stimulation of interferon production (8*a*, 11, 14, 22). The interferon-blocking viruses, in the instances reported, either did not elicit interferon themselves or, at best, produced low yields of interferon. Furthermore, blockage of interferon synthesis was shown to facilitate and enhance replication of the second virus (8*a*, 11). In all tests, pretreatment of cultures with the interferon-blocking virus nullified the antiviral effect of subsequently added exogenous interferon (8*a*, 11). A comparable state of nonresponsiveness to interferon induction can be detected in cells which have been exposed to inactivated, interferon-producing viruses incapable of replication (4, 6) and to statolon (29). Cells developed resistance to viral superinfection as well as to stimulation of interferon formation, but in growing cultures refractoriness was lost well in advance of protection (6, 27). Cultures were thus obtained which were perfectly capable of elaborating normal amounts of interferon on appropriate stimulation although still unable to support virus growth.

Kinetic studies on the production of interferon and the development of refractoriness in L cells disclosed that nonresponsiveness to interferon induction was detected concurrently with the deceleration of interferon production stimulated by irradiated NDV (29). This finding suggested that these two cellular events may be more than coincidental occurrences. Speculation was further stimulated by the observation that the interferon-containing medium collected from such noninfectible and refractory cells was not only endowed with antiviral activity, but that, after elimination of any residual virus present, it was also capable of depressing the cellular response to stimulation of interferon production (6, 35). Furthermore, the refractoriness-inducing effect was not duplicated in mouse cells by interferons derived from other species, and it was fully neutralized by specific anti-interferon serum (K. Paucker, Bacteriol. Proc., p. 119, 1966). The interferon-depressing property displayed, therefore, the same high degree of host specificity which characterized the antiviral capacity of interferon as shown with purified materials (21, 26) and by immunological methods (28). It was tentatively concluded that, in analogy with some bacterial systems, the accumulation of interferon as an end product of host-virus interaction may function as a depressant of further production and release of interferon and, in addition, deter attempts at restimulation. However, on further study, a certain amount of circumstantial evidence accumulated which suggested that the antiviral and interferon-depressing characteristics in interferon materials are, indeed, dissociable. The excessive doses of interferon required to elicit refractoriness, the temporal aspects governing the development and loss of resistance to superinfection and interferon stimulation, the widely divergent adsorption kinetics of these properties, and, finally, a certain degree of separation on partial purification all pointed to the fact that two distinct factors might be involved in these phenomena (K. Paucker, Bacteriol. Proc., p. 119, 1966).

Efforts at isolation of a component in L-cell interferon preparations which renders cells refractory without affecting their infectibility by viruses have not yet been successful. So far, it is known that this property withstands the methods used routinely for preparation of interferon, i.e., treatment with perchloric acid (20), exposure to *p*H 2 (5), and precipitation by zinc acetate (20) or by ammonium sulfate (3). The effect is nondialyzable and cannot be demonstrated in L cells with interferons derived from other species; it is neutralizable by specific anti-interferon serum but not by immune serum against the virus used in preparation of the interferon (*unpublished data*).

It resembles in many of these respects the extraviral "blocker" of interferon production which is found in allantoic fluids of embryonated eggs infected with some myxoviruses and in tissue culture fluids from cells infected with Chicungunya virus (15*a*). The blocking factor shares also many of the physical characteristics of interferon, except for its stability in the presence of proteolytic enzymes. This property permitted the selective elimination of interferon and study of the activity of the blocker unencumbered by viral interference. It was found to exert no effect on the action of exogenous interferon and to promote enhanced growth of Chicungunya virus, presumably by virtue of its capacity to inhibit interferon production by this virus.

The enhancing factor of Kato et al. (16, 17), which precedes the appearance of interferon in allantoic fluids of eggs inoculated with Sendai or PR8 influenza viruses, may be similar in nature. However, the effect of the "enhancer" on the production of interferon is not yet known. It appears to be capable of inhibiting the activity of

exogenous interferon under certain conditions (17*a*).

Nonviral substances which affect the behavior of cells with respect to interferon action have also been elicited by viruses which do not themselves give rise to interferon formation (2, 8, 9). Their relationship to the findings discussed in this paper as well as to the above-mentioned factors is not clear. The inhibitor of Ghendon (9) interferes with the action, but not with the production, of interferon in chick embryo fibroblasts infected with Newcastle disease virus, and it was proposed that this activity accounts for the increased multiplication of the virus. "Stimulon" (8) designates a substance, produced in human embryonic kidney cells infected with adenovirus 12, which enhances the replication of Kilham's rat virus in rat embryo cells. It counteracts the effect of Sendai-induced interferon but not the production of interferon by the same virus (8). Unlike the depressor of interferon formation in L cells, stimulon operates in the heterologous species and is neutralized by antiviral serum (2). In contradistinction to blocker, stimulon is digested by trypsin (8).

On the basis of the data presented and of studies in other laboratories, the following general conclusions seem to be warranted. Incident to viral multiplication, various substances are produced which can affect cellular behavior with respect to interferon in different ways. There are those extraviral components which inhibit only the action of interferon but not its formation. They do not appear to be involved in cell regulatory mechanisms of interferon production, and they may be considered as belonging to a general class of substances which promote the multiplication of certain viruses susceptible to their effect (8). Other factors are capable of suppressing the production of interferon. These may be regarded as regulators of interferon synthesis in the cells in which they were produced. The studies carried out in the NDV-L cell system have shown that cultures in which interferon formation has ceased are refractory to further stimulation of interferon production. It is suggested that the synthesis of interferon in these cells is accompanied at some stage by the appearance of a repressor which blocks further production of interferon. The repressor, like interferon, may be released into the culture fluid, and is presumed to be responsible for the refractoriness-inducing effect originally ascribed to interferon.

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