Advances in VIRUS RESEARCH

Edited by

KENNETH M. SMITH MAX A. LAUFFER
Department of Biophysics
University of Pittsburgh
Pittsburgh, Pennsylvania

VOLUME 10

1963
ACADEMIC PRESS
NEW YORK AND LONDON

ACADEMIC PRESS INC.

111 FIFTH AVENUE

NEW YORK 3, N. Y.

United Kingdom Edition

Published by

ACADEMIC PRESS INC. (LONDON) LTD.

BERKELEY SQUARE HOUSE, LONDON, W. 1

Library of Congress Catalog Card Number 53-11559

PRINTED IN THE UNITED STATES OF AMERICA

CONTRIBUTORS TO VOLUME 10

SAMUEL BARON, *Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare, Bethesda, Maryland*

IRWIN J. BENDET, *Department of Biophysics, University of Pittsburgh, Pittsburgh, Pennsylvania*

R. W. HORNE, *Institute of Animal Physiology, Babraham, Cambridge, and Medical Research Council Unit for Experimental Virus Research, Institute of Virology, University of Glasgow, Scotland*

ALICK ISAACS, *National Institute for Medical Research, Mill Hill, London, England*

B. KASSANIS, *Rothamsted Experimental Station, Harpenden, England*

W. C. PRICE, *Plant Virus Laboratory, University of Florida, Gainesville, Florida*

P. WILDY,* *Institute of Animal Physiology, Babraham, Cambridge, and Medical Research Council Unit for Experimental Virus Research, Institute of Virology, University of Glasgow, Scotland*

* Present address: University of Birmingham, England.

CONTENTS

Inactivation and Denaturation of Plant Viruses

W. C. Price

Interactions of Viruses in Plants

B. Kassanis

INTERFERON

Alick Isaacs

National Institute for Medical Research, Mill Hill, London, England

I. Introduction

A. Definition

Interferon is the name that was given to an antiviral substance produced by the cells of many vertebrates in response to virus infection. It appears to be of protein or polypeptide nature, it is antigenically distinct from virus, and it acts by conferring on cells resistance to the multiplication of a number of different viruses.

B. Viral Inhibitory Substances Recovered from Virus Infections

Interferon derived its name from virus interference, since it was first isolated and characterized during a study of this phenomenon (Isaacs and Lindenmann, 1957). However, similar substances were previously observed, although they were not characterized. Ørskov and Andersen (1938) found that within a short time of the initiation of vaccinial infection of the rabbit skin local "antibody" could be detected at the site of infection at a time when none could be found in the serum. In retrospect, Ørskov (personal communication, 1962) feels that this was interferon, not antibody. Gard (1944) studied tissue immunity in mouse encephalomyelitis and observed an interfering factor that was separable from the virus. He found that a suspension of brain from mice infected with Theiler's mouse encephalomyelitis virus was able to inhibit the growth of virulent virus in fresh mice; the inhibitory factor seemed to be cell-bound and did not act by combining with the challenge virus. Lennette and Koprowski (1946) found that infected cultures of chick and mouse embryo tissue when freed of virus showed a very weak viral inhibitory action which they thought could not explain the viral interference they observed. Nagano and Kojima (1954) studied a similar experimental situation to that of Ørskov and Andersen (1938) and they also found a virus-inhibitory substance separable from the infecting virus in extracts of infected rabbit skin. However, these authors were unable to decide whether the inhibition found was an immunological or an interference effect; indeed it is difficult in experiments in animals to distinguish how much of the viral inhibitory action found might be due to specific antibody, to inactivated virus or viral antigens capable of inducing virus interference, or to interferon. Thus the recent experiments of Matumoto *et al.* (1959) show that infection of mice with neurotropic Rift Valley fever virus protects them against the virulent pantropic variant; protection is slight if the two viruses are injected together but increases the longer the interval between the two. Their results suggest that viral interference may have played a more important role in inducing protection when the two viruses were injected together, but with lengthening interval of time between the two, specific immunity may have become more important. To distinguish these it has become necessary to carry out experiments in chick embryos or in tissue culture, in order to exclude antibody, and to allow adequate characterization of the virus inhibitory substances found.

In this review, no attempt will be made to summarize studies on virus interference which have been thoroughly covered by Henle (1950), Schlesinger (1959), and Wagner (1960). Nor will any attempt be

made to evaluate the role of interferon in virus interference beyond drawing attention to the evidence described by Henle *et al.* (1959) and Isaacs (1959) that many examples of virus interference can be accounted for by the production of interferon by cells in response to contact with the interfering virus. Although work on interferon began as an attempt to find an explanation of viral interference, an early observation was that once formed, interferon was rapidly liberated from cells and could be found in much higher concentration in the extracellular fluid than within cells (Isaacs and Lindenmann, 1957; Vilček, 1961; Bader, 1962). This raised the possibility that interferon was capable of protecting not only the cells initially infected but also neighboring cells. Thus, attention was soon directed toward considering the possible role of interferon in cellular resistance to virus infection, in general, and in the processes of recovery from virus infection in particular. These themes will therefore be dealt with in Section VI of this review in place of a consideration of virus interference.

C. Techniques of Assaying Interferon

Many different techniques are used to assay interferon. The most generally used, however, measure the degree of inhibition in the ability of treated cells to produce virus after infection. This is measured either as a diminution of the yield of virus from treated cells or as a diminution in the abililty of treated cells, when infected, to initiate the production of a viral lesion (e.g., a plaque in cell monolayers). The technique which was first used (Isaacs and Lindenmann, 1957) was to measure the reduction in the yield of influenza virus hemagglutinin from pieces of chick chorioallantoic membrane infected *in vitro* by the technique of Fulton and Armitage (1951). This method was based on the finding of a linear relationship between the degree of virus inhibition produced and the concentration of interferon used (Isaacs *et al.*, 1957). This type of method has now been largely replaced by a plaque assay method in which the concentration of interferon that will produce a 50% reduction in the plaque count in a cell monolayer is measured (Wagner, 1960). In assays with different experimental systems a linear relation has been found between the degree of reduction of the plaque count and the logarithm of the concentration of interferon over quite a wide range of concentrations, so that the end point of the assay can, if necessary, be determined by interpolation. Gifford *et al.* (1963) have developed an assay of this kind based on the method of Postlethwaite (1960) for producing plaques with vaccinia virus without using an agar overlay. When the logarithm of interferon concentration was plotted against the reduction in plaque count, an S-shaped curve was formed which

was linear over a certain range of concentrations of interferon. They also found a linear relationship when the relative average plaque diameter or the total plaque area was plotted against the logarithm of interferon concentration. A third method of assay is based on the size of the zone of protection produced in a sheet of virus-infected cells when a cup containing interferon is placed over the agar overlay. Porterfield (1959) showed that there was a linear relationship between the concentration of interferon, plotted logarithmically, and the area of the protected zone. Another technique used is to measure the degree to which a culture of cells is protected against the cytopathic action of virus as judged microscopically (Sellers and Fitzpatrick, 1962). This assay gives a linear relationship between the logarithm of the concentration of interferon and the logarithm of the amount of virus inhibited. Sueltenfuss and Pollard (1963) have developed a very sensitive assay which is based on inhibition of the development of the inclusions produced by psittacosis virus, as judged by fluorescence microscopy of cells stained with acridine orange. These are the basic methods most commonly used in assaying interferon; the review by Porterfield (1963) gives a more detailed description of the techniques used.

II. Production by Different Cells and Viruses

Production of interferon was studied first in chick cells infected with inactivated influenza virus, but it soon became clear that similar substances were produced by the cells of many animal species in response to infection with a variety of different viruses.

A. Production by Cells of Different Animal Species

Among the animal species whose cells have been shown to produce interferon *in vitro* are chickens, ducks, mice, rats, guinea pigs, hamsters, rabbits, ferrets, dogs, sheep, pigs, cows, monkeys, and man. Table I of the review by Ho (1962b) gives many references to work describing production of interferon by different cell-virus systems. Production during the course of infection *in vivo* has been demonstrated in chick embryos, mice, and rabbits, but has been much less studied than production *in vitro*.

The fact that birds produce interferon raises the question of how early in evolution such a mechanism might have arisen. Virus interference has been found among bacterial and plant viruses but it is not known whether it is mediated by substances similar to interferon, although one report has appeared indicating that an interferon-like substance was produced by *Pseudomonas aeruginosa* infected with

bacteriophage (Mercer and Mills, 1960). The nature of the repressor that is responsible for some cases of immunity to superinfection shown by lysogenic bacteria (Jacob, 1959) is not yet known, but the fact that the immunity tends to be specific toward the infecting phage does not favor the suggestion that the repressor might function in the same way as interferon.

B. Production by Different Varieties of Cells

No systematic study has been made of the production of interferon by cells from different organs, but no striking differences in the behavior of cells have been found in *in vitro* or *in vivo* studies. Thus, *in vivo*, production of interferon has been observed in the mouse brain and lungs and in the rabbit skin, and *in vitro*, in chick chorionic and allantoic cells, human amnion cells, calf, dog, monkey, and human kidney cells, human thyroid cells, and human leucocytes. Until now, no differences have been observed between the behavior of epithelial cells or fibroblasts.

Certain lines of tumor cells were thought at first to be poor producers of interferon (e.g., Henle *et al.*, 1959) but this may be due to the fact that many tumor cell lines are very insensitive to the antiviral action of interferon, even to that produced in the same cells. Thus, Ho and Enders (1959a,b) found that HeLa cells produced interferon which they could assay on primary human amnion cells but not in HeLa cells. Similar findings were reported for KB cells by Chany (1961), for HeLa cells by Vilček (1962), and for a human amnion cell line by Mayer (1962). However, this is not an invariable finding since Cantell (1961a) and Isaacs *et al.* (1961b) have found that certain lines of HeLa cells show some sensitivity to the action of interferon, although less than that of primary human thyroid cells, in the case of one cell line studied. It was suggested by Isaacs *et al.* (1961b) that this behavior of tumor cells might reflect metabolic differences from normal cells, and it would be interesting to study this question in lines of HeLa cells differing in sensitivity to interferon.

Embryonic cells have been used extensively to produce interferon, but chorioallantoic cells of 6-day chick embryos were found to produce only about one-tenth as much interferon as the cells of 11-day embryos after treatment with irradiated influenza virus (Isaacs and Baron, 1960). Also suckling mice infected intranasally during the first day of life with parainfluenza 1 (Sendai) virus produced more virus but less interferon than did 4-week old mice similarly infected (Sawicki, 1961). The question of whether it might generally be found that cells show

increased production of interferon with aging of the animal of their origin, or aging *in vitro*, requires further investigation.

C. Production of Interferon by Inactivated Virus

First studies of interferon were carried out with inactivated myxoviruses. Among the viruses shown to produce interferon were influenza A and B, Newcastle disease, and fowl plague viruses, inactivated by irradiation with ultraviolet (UV) light, heating at 56° or at 37°C., but not by treatment with formaldehyde (Burke and Isaacs, 1958b). Other viruses shown to induce the production of interferon when used inactivated are mumps (Cantell, 1961a), Rous sarcoma virus (Bader, 1962), vaccinia (Glasgow and Habel, 1962), and herpes simplex (Waddell, 1962). Incomplete influenza virus, produced by repeated passage at high virus concentration, has been shown to induce interference (von Magnus, 1954) and to induce production of interferon when inoculated on the chick chorion at a site where virus multiplication does not occur (Burke and Isaacs, 1958a). Interferon induced by different viruses shows no evidence of specificity, i.e., it is not most active when tested against the homologous virus (Lindenmann *et al.*, 1957).

Ho and Breinig (1962) have found that Sindbis virus heated at 56°C. for 4 hours did not induce production of interferon but was able to "sensitize" cells so that they now produced interferon when infected with live Sindbis virus. A number of reports have appeared indicating absence of interferon production by arboviruses and enteroviruses when used inactivated (e.g., Ho and Enders, 1959b). With one arbovirus, inactivation by deoxycholate was found to produce a virus still capable of inducing interference but no interferon could be detected (Henderson and Taylor, 1961). However, the fact that interferon was not detected makes it difficult to conclude that none was produced since the conventional tests measure only excess interferon liberated from cells. Before concluding that a virus once inactivated does not produce interferon it will be necessary to examine different types of inactivation, since it is known that if influenza virus is heated too much (Isaacs and Lindenmann, 1957) or over-irradiated (Burke and Isaacs, 1958a), it loses its ability to produce interferon. The results of Ho and Breinig suggest that, at least with one virus, prolonged heating may have reduced its ability to stimulate the production of good titers of extracellular interferon while retaining its ability to sensitize cells to respond to infection by live virus by producing interferon. Influenza virus more gently inactivated by heat was able to induce production of interferon and was found to sensitize cells to respond to infection by live virus by producing a rapid synthesis of interferon (Burke and Isaacs, 1958b).

Recently, Vilček (1963) has studied production of interferon in

chick cells induced by tick-borne encephalitis virus inactivated by incubation for various periods of time at 37°C. He has concluded that interferon production could be demonstrated only when live virus was present. Vilček points to the fact that among viruses that have been shown to induce production of interferon when used in the inactivated form it has not yet proved possible to obtain infective viral nucleic acid. Alternatively, in the viruses among which, until now, no clear evidence of production of interferon by inactivated viruses has so far been shown, it is readily possible to prepare infective viral RNA (ribonucleic acid). This seems to be an interesting division among viruses, although so far its significance is unknown.*

The findings quoted above concern production of interferon by virus which has been rendered noninfective by a particular treatment. The converse situation is infection by live virus of cells that are "insusceptible," implying that the cells are unable to support a complete cycle of growth by a particular virus. Interferon production of this kind has been found with influenza virus in chick chorionic cells (Lindenmann *et al.*, 1957) and by parainfluenza 1 and measles viruses in human leucocytes (Gresser, 1961b). It seems clear, therefore, that virus multiplication is not essential for production of interferon. The question of which viral constituent stimulates cells to produce interferon will be discussed in Section VII.

D. Production of Interferon by Live Virus

The term "live virus" is used to denote virus prepared in such a way as to avoid as much as possible any loss of infectivity. However, with animal viruses kept under optimal conditions, the majority of the virus particles are incapable of initiating infection, the ratio of infective particles to total virus particles being usually of the order of 1 to 10. Since some strains of influenza virus grown in suspended chick chorioallantoic membranes gave rise to good yields of interferon within 6–12 hours of infection with inactivated virus, and poorer yields of interferon at a later stage of infection with live virus, it is possible that production of interferon by live virus is due largely to particles in the virus population that are not undergoing multiplication. This question cannot be resolved until methods are available for measuring the yield of interferon from single cells. It is discussed further in Sections II,F and VI.

The review by Ho (1962b) gives in Table I a list of references to production of interferon by different live viruses. Viruses shown to

* However, Gifford and Heller (1963) have now found good yields of interferon on infecting chick cells with an arbovirus (Chikungunya virus) inactivated by incubation for 23 hours at 35°C.

induce production of interferon include RNA and DNA (deoxyribonucleic acid) viruses, all ranges of size from foot-and-mouth disease virus (Dinter, 1960) to the pox viruses (Nagano and Kojima, 1958), cytolytic viruses, e.g., arboviruses, and tumor viruses, e.g., polyoma (Allison, 1961). It seems justifiable to conclude, therefore, that production of interferon is a very general response of cells to virus infection. The yield of interferon differs greatly with different viruses grown in the same cells or with a single virus grown in different cells. This is discussed further in Section VI in relation to the problem of virus virulence.

E. Sensitivity of Different Viruses to the Antiviral Action of Interferon

In addition to the differences in the yield of interferon which they can induce, viruses also differ in their sensitivity to the antiviral action of interferon on cells. The two properties give the impression of being related, since it is frequently found that viruses that give good yields of interferon are sensitive to its antiviral action, and conversely, that viruses that give poor yields of interferon are generally much less sensitive to its antiviral action. It is not known whether there is any necessary relationship between these two properties. Possibly tests of the sensitivity of a virus to interferon measure indirectly the probability that a particle belonging to a particular virus population will induce the production of interferon instead of virus, in both normal cells and interferon-treated cells.

Differences found in the sensitivity to the antiviral action of interferon may be quite considerable. Thus roughly 30 times more interferon was required to cause 50% inhibition of plaque production by Newcastle disease virus than by O'nyong-nyong virus grown in chick embryo fibroblasts (Ruiz-Gomez and Isaacs, 1963a). An early observation was that herpes simplex virus was much more resistant to the action of interferon than vaccinia or cow pox viruses grown on the chick chorion (Isaacs *et al.*, 1958). Ho and Enders (1959b) found that herpes simplex virus was much more resistant to interferon than vaccinia or Sindbis viruses grown in human amnion or human kidney cells. Relative resistance of herpes simplex was also observed by Cantell and Tommila (1960) in the rabbit cornea and by Vilček and Rada (1962) in chick embryonic cells, and the closely related pseudorabies virus was shown to behave similarly by Vilček (1962) and by Dinter and Philipson (1962). Adenovirus type 7 was found to be very resistant to the action of interferon in HeLa cells (Cantell, 1961a). Viruses that have been shown to be relatively resistant to the action of interferon include strains of fowl plague, Newcastle disease, herpes simplex, pseudo-

rabies, and adenovirus. However, Glasgow and Habel (1962) found herpes simplex virus relatively sensitive to the action of interferon in mouse cells; it is not known whether this is due to the use of a different strain of virus, or different cells from those used by other workers.

As a general rule, vaccinia virus and many arboviruses and rhinoviruses seem to be relatively much more sensitive to the antiviral action of interferon (Baron *et al.*, 1961; Sutton and Tyrrell, 1961) although differences in sensitivity among the arboviruses can be shown. Differences in sensitivity among different viruses have been related to differences in oxygen requirement, in optimal temperature for virus growth, and in virus virulence. These points are discussed in the following sections since they may throw some light on the mode of action of interferon.

F. Factors Concerned with the Production of Interferon

Production of good yields of interferon was observed within 6 hours of infection of chick chorioallantoic membrane with heated influenza virus (Isaacs and Lindenmann, 1957) or infection of human leucocytes with parainfluenza 1 virus (Gresser, 1961b), which does not multiply in these cells. Incubation at about 37°C. was required, incubation at 2°C. giving no significant yield of interferon. Production of interferon continued for 24 hours when it gradually ceased, but a second inoculation of heated influenza virus at this time gave rise to a second crop of interferon (Lindenmann *et al.*, 1957). Irradiated influenza virus gave a more long-lasting stimulus, production of interferon being detectable in small amount even on the third day after infection (Burke and Isaacs, 1958b). The fact that protein synthesis is required for the production of interferon is indicated by the inhibition of interferon formation produced by treating cells with *p*-fluorophenylalanine (unpublished observations).

Within a short time of being detected within cells interferon was rapidly liberated and was recovered in good yield from the suspending medium (Isaacs and Lindenmann, 1957). Interferon produced in chorionic cells could be shown to diffuse not only outward from the chorionic surface but also inward through the mesoderm to the allantoic cells (Isaacs *et al.*, 1958). This rapid liberation from cells first suggested that interferon might be capable of protecting not only the cells initially infected but also neighboring cells.

Early production and rapid liberation of interferon is characteristic of infection with inactivated or nonmultiplying virus. However, following infection by live virus of cells able to support virus multiplication, interferon is usually detectable only after some delay. When chick chorioallantoic membranes were infected with a large dose of influenza

virus, multiplication of virus occurred within the first 24 hours but no interferon was detected. During the next 24 hours virus multiplication slowed down and interferon was then produced (Burke and Isaacs, 1958b). Wagner (1960) has shown growth curves of influenza virus cultivated in the chick embryo in which interferon appeared in the allantoic fluid 24 hours after the production of viral hemagglutinin. Production of interferon occurring 24–48 hours after virus production was also observed for infection of the mouse brain with O'nyong-nyong virus (Hitchcock and Porterfield, 1961) and infection of chick cells with tick-borne encephalitis virus (Vilček, 1961). This delay following infection with multiplying viruses contrasts with the early production of interferon when inactivated or nonmultiplying virus is used. One possibility is that the delay allows time for virus inactivation to occur and that the inactivated virus then sets off the production of interferon. Alternatively, it is possible that an individual cell actively supporting virus multiplication produces no interferon until a late stage of virus multiplication is reached, when interferon may then accumulate and help to bring virus production to a halt.

III. Properties

A. Physicochemical Properties

Interferon is nondialyzable and not sedimented on centrifugation at 100,000 *g* for 4 hours (Isaacs *et al.*, 1957). Estimates of its molecular weight have been based on its rate of diffusion and its behavior on centrifugation. Porterfield *et al.* (1960) measured the size of the zones of protection produced in virus-infected chick cells to which were applied at various intervals of time after infection beads containing either chick interferon or viral antibody. The diffusion coefficient of interferon was found to be much higher than that of rabbit antibody and the molecular weight was estimated as less than 80,000. Burke (1961) studied the behavior of purified chick interferon in the analytic ultracentrifuge. The interferon behaved in the ultracentrifuge as a single component with a molecular weight of 63,000 and a sedimentation constant of 4.77 S. Little work has been reported on the molecular weight of interferons of other animal species, although they resemble chick interferon in being nondialyzable and not sedimented at 100,000 *g* for periods of 1–2 hours.

Note added in proof: Recently, new information has appeared on the molecular weight of interferon. Lampson *et al.* (1963) studied a highly purified preparation of chick interferon and estimated, by means of

high-speed centrifugation, that it had a molecular weight of 20,000–34,000. Rotem and Charlwood (1963) carried out studies of the molecular weight of chicken, mouse, and monkey interferons by means of sedimentation in sucrose density gradients along with radioactive-labeled markers of known molecular weight. By use of this technique, all three interferons were found each to have a molecular weight close to that of lysozyme with limits of 13,000–25,000. It seems likely from these findings that the preparation studied by Burke cannot have been purified sufficiently.

The protein, glycoprotein, or polypeptide nature of interferon is inferred primarily from the fact that its antiviral activity is greatly reduced or abolished by treatment with proteolytic enzymes, e.g., trypsin (Lindenmann *et al.*, 1957), pepsin (Burke and Isaacs, 1958a), or chymotrypsin (Wagner, 1960). On the other hand, it was not affected by treatment with ribonuclease, deoxyribonuclease, or neuraminidase. Some of its other physicochemical properties are those that might be expected of a protein. According to Lampson *et al.* (1963), one unit of interferon activity in an assay in chick cells was 0.0042 μg. of protein.

Interferon is stable on storage at 2°, —10°, or —70°C. However, the reports of the stability of interferon on heating have been very conflicting. Chick interferon was inactivated on boiling for 5 minutes. In an early report it was found to be inactivated on heating at 60°C. for 1 hour (Isaacs *et al.*, 1957) but this result may have been due to the pH not having been controlled. On heating at pH 7.2 to 7.4 it resisted heating at 60°C. for 1 hour (Isaacs, 1960b). Wagner (1960) found interferon prepared from chick allantoic fluid to resist heating at 70°C. for 1 hour, and it is possible that other proteins present in the allantoic fluid may stabilize the interferon to heat. Human interferon was found to have its activity reduced but not abolished by heating at 56°C. for 30 minutes (Ho and Enders, 1959a) and to be completely inactivated by heating for 1 hour at 60°C. at pH 7.8 (Gresser, 1961a), a finding which corresponds to our experience with human interferon. On the other hand, Chany (1961) found human interferon to be completely inactivated at 56°C. for 30 minutes, whereas Mayer (1962) found it to be stable on heating at 60°C. for 1–2 hours. Rabbit interferon was found to resist heating at 56°C. for 30 minutes but to lose activity on heating at 65°C. (Nagano and Kojima, 1958). Mouse interferon was found to be more heat-labile than chick interferon, being inactivated by 60°C. for 1 hour (Henle *et al.*, 1959; Isaacs and Hitchcock, 1960), whereas Glasgow and Habel (1962) reported mouse interferon to be stable after heating at 60°C. for 1 hour. In view of the biological differences in interferons from different animal species discussed below,

it would not be surprising if they differed in heat stability too, as occurs, for example, in the case of ribonucleases from different sources. However, some of the conflicting reports raise the question of the influence of other constituents present along with the test materials on the apparent heat stability of interferon.

Interferon is stable over a wide pH range, from pH 1–10 (Lindenmann *et al.*, 1957). It is also very stable on irradiation with UV light (Burke and Isaacs, 1958a; Nagano and Kojima, 1958; Zemla and Vilček, 1961b).* It can be precipitated by saturated ammonium sulfate (Lindenmann *et al.*, 1957) or by acetone or ethanol (Zemla and Vilček, 1961a,b). Its reported behavior with ether seems to be variable.

Most of the reported investigations have been concerned with chick interferon. More investigation is required to know whether interferons from other animal species have similar physicochemical properties.

B. Biological Properties

1. Antigenicity

Interferon is antigenically quite distinct from the virus that induced its production (Isaacs *et al.*, 1957). This is such a fundamental point of distinction that it has been included in the definition given at the beginning of this chapter.

Interferon appears to be a poor antigen. When inoculated into rabbits or hens either alone or with oil adjuvants or after precipitation with alum, chick interferon did not induce the production of neutralizing antibody (Burke and Isaacs, 1960; Lindenmann, 1960) nor of precipitating antibody (Belton, personal communication, 1960). Nagano and Kojima (1960) found that a series of injections of rabbit interferon into hens, guinea pigs, and two groups of rabbits produced no neutralizing antibodies; however, a third group of rabbits developed neutralizing antibodies as measured in the rabbit skin. Later Nagano and Kojima (1961) confirmed this finding and also found neutralizing substances in the serum of immunized fowls. Recently Paucker and Cantell (1962) have found that after prolonged immunization of guinea pigs with mouse interferon a very low-titered antibody was found. Antibody could be demonstrated only by using very dilute preparations of interferon. As far as the evidence goes, therefore, interferon appears to be a very weak antigen.

The fact that interferon is quite distinct from virus serologically allows the use of viral antibody to inactivate virus without affecting

* Lampson *et al.* (1963) do not find this to be true of highly purified chick interferon.