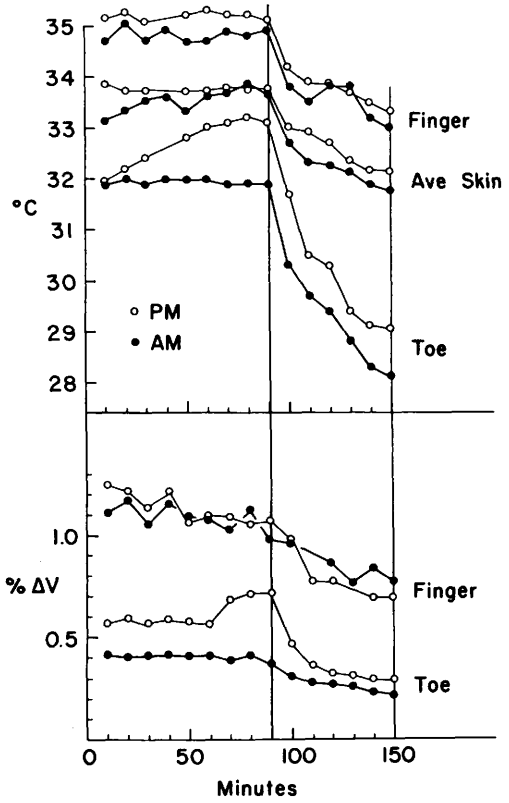

FIG. 1. Temperature and digital pulse during exposure at 30°C (first 90 minutes) and at 25°C (following 90 minutes). Open circles are data from afternoon experiments, closed circles are morning experiments.

with Newton's Law of cooling ($H = K(T_s - T_a)$ where H is the heat lost, T_s skin temperature and T_a the ambient temperature, the conclusion might be drawn that the shift in T_s should be the same as the shift in T_a (*i.e.* 5°C) since K should not change and H might be assumed to be constant. That this is not the case is readily apparent and since T_s does not change by 5°C, H must be altered by an internal mechanism.

When the finger blood flow, as indicated by the %ΔV/beat(4), is plotted against mean skin temperature the values show the tendency for the morning and afternoon values to be displaced, the afternoon values being higher. The slope however is not different. A similar plot for the toe blood flow suggests a slope change.

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Relationship of Interferon Production to Virus Growth *in vivo*. (29575)

SAMUEL BARON, HERMAN G. DU BUY, CHARLES E. BUCKLER AND MARTIN L. JOHNSON

U. S. Department of HEW, USPHS, Nat. Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Biology Viruses, Bethesda, Md.

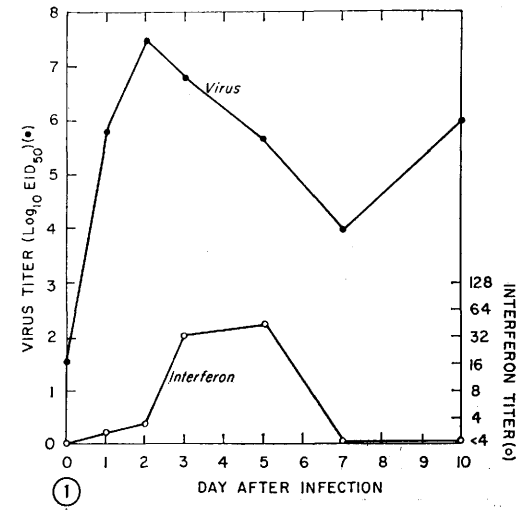
Interferon has been implicated as an important host factor which contributes towards recovery of animals from virus infection(1, 2). Early studies of interferon production in infected organs indicated a close correlation with virus growth(3,4,5). The present study was undertaken to compare the relationship of interferon production with virus growth during infection of mice with various viruses. The results indicate that interferon production may decline during the later stages of certain virus infections despite continued growth of virus.

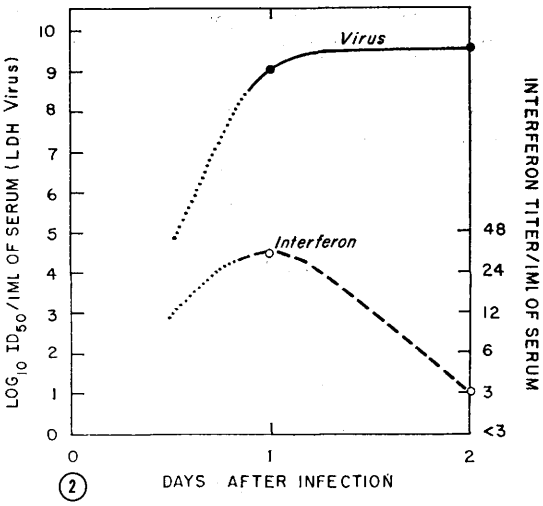
Materials and methods. Interferon was assayed as described previously(6). It was characterized through its activity against heterologous virus, by: lack of activity in host cells from heterologous species, inactivation

with crystalline trypsin, inability to be sedimented at 105,000 × gravity for at least 2 hours and stability at pH 2. The PR8 strain of influenza virus type A was propagated and assayed for infectivity in 11-day-old embryonated eggs. The small plaque forming variant of encephalomyocarditis virus (EMC) was obtained from Dr. K. K. Takemoto and plaque assayed for infectivity in the presence of DEAE-dextran(7). Lactic dehydrogenase virus (LDH) was prepared as a mouse serum pool and assayed for infectivity by injecting 10-fold serial dilutions into 5 mice per dilution. Blood was collected 4 days after inoculation by orbital bleeding and pooled. LDH enzyme activity was determined on each pool by a modification of the method of Wroblewski and La Due(8). LDH virus titer was expressed as the negative $\log_{10}$ dilution of sample which induced increased LDH enzyme in 50% of the mice (ID_{50}). NIH general purpose strain of Swiss mice were used throughout. Fifteen gram males were used except for LDH experiments where 25 g females were used. Each point in the figures was determined by appropriate assays of pooled serum or lung extracts obtained from 5 individual mice. Except for the orbital bleedings in the LDH experiments, mice were exsanguinated through an incision in the axilla.

Results. To determine the relationship of interferon production to virus growth during non-fatal viral pneumonia, mice were infected intranasally with 100 50% egg infectious doses of PR 8 virus and lung extracts assayed for virus and interferon. These mice were treated with Thiotepa to delay antibody production, and, consequently, it was possible to observe virus growth in the absence of interfering antibody(9). Although interferon production occurred shortly after virus growth, no interferon was detectable after the 5th day despite high virus titers (Fig. 1).

Similar divergence of interferon production





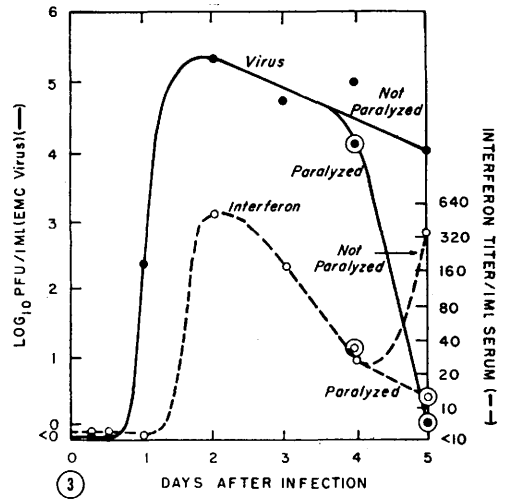


FIG. 1. Interferon and virus titers in lungs of mice infected with PR8 strain of influenza virus.

FIG. 2. Interferon and virus titers in sera of mice infected with LDH virus.

FIG. 3. Interferon and virus titers in sera of mice infected with EMC virus.

and virus growth in serum was observed during infection of mice with LDH virus. Mice were infected intraperitoneally (IP) with $10^{6.0}$ ID_{50} LDH virus and bled after 24 and 48 hours for virus and interferon assays of serum. There was a rapid rise of virus in the serum over the 2-day period (Fig. 2). Interferon titer was highest at 24 hours and declined at 48 hours. As late as 14 days after infection no interferon was detected in the serum although virus persisted at $10^{6.0}$ ID_{50} per ml.

In analogous experiments, using lethal arbovirus infections of mice, circulating interferon was observed to decline despite continued high viremias(10). Also during vaccinia virus infections of the skin of guinea pigs, interferon titers were observed to decline as the infection progressed although virus titers remained constant(11).

A different relationship of interferon production to virus growth was observed in mice infected IP with a 70% lethal dose of EMC. Serum interferon production closely paralleled the viremia throughout the experiment (Fig. 3). In those mice in which virus declined there was a corresponding decrease in interferon. Conversely, when viremic levels remained high, interferon production persisted. This close relationship of interferon production to virus growth is similar to that observed in earlier studies with certain influenza and arboviruses(3,4,5).

Discussion. The present results indicate that measurable interferon production does not persist during the later stages of certain virus infections. A confirmatory finding is that interferon production has also been observed to decline during polyoma infection of mice despite continued growth of virus(12). These observations, especially those with LDH infection, may help explain the reported inability to detect interferon production during a chronic infection of mice with lymphocytic choriomeningitis virus(13). Similarly, the inability to detect interferon in the lungs of persons who died of influenza pneumonia (14) may be due to lack of interferon production during the later stages of infection.

The reasons for the failure of virus to continue to induce interferon production are speculative. Perhaps the explanation is related to the observation that cells which have absorbed or produced large amounts of interferon do not produce interferon when subsequently infected(15,16). Also it is known that interferon which has been taken up by cells cannot be extracted in active form (17). Thus, the interferon which is produced only during the early stages of these virus infections might be absorbed and masked within cells and subsequently tend to inhibit production of interferon after infection. We favor the possibility that many cells may be producing small amounts of interferon during the later stages of infections but these small amounts of interferon are held within the producing cell. This interpretation is in accord with the hypothesis that some types of viral interference, in the absence of detectable interferon, are probably due to undetectable amounts of intracellular interferon (18). Such possible interpretations still fail to account for all the observed phenomena, *e.g.*, continued virus growth later in infection. However, interferon may still participate in the later stages of recovery despite the absence of its continued production since the antiviral action of absorbed interferon may be persistent for a number of days(19,20,21).

Summary. Interferon production and virus growth were followed during infections of mice. Although interferon production paralleled encephalomyocarditis virus titers in the serum, interferon production did not parallel virus growth during the later stages of influenza infection of mouse lung nor did it parallel lactic dehydrogenase virus levels in the sera of infected mice.

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Heat Stability of Avidin and Avidin-Biotin Complex and Influence of Ionic Strength on Affinity of Avidin for Biotin.* (29576)

RU-DONG WEI† AND LEMUEL D. WRIGHT

Graduate School of Nutrition, Cornell University, Ithaca, N. Y.

Avidin, a toxic protein, which renders biotin unavailable for animal and microbial growth combines firmly with biotin to yield an avidin-biotin complex with a dissociation constant of 10^{-15} M(1). Biotin, when combined with avidin, cannot be liberated from the avidin-biotin complex by ordinary proteolytic enzymes(2), and the complex is stable over a wide pH range(3,4). It has been reported, however, that bound biotin is completely released from the complex by steam sterilization(3). The microbiological assay (5) of avidin is based on this property. In a study of a new method for determination of avidin by use of radioactive biotin and gel-filtration, conducted by the present authors (6), it was discovered that the so-called heat labile complex is quite stable in 0.2 M ammonium carbonate solution. For example, it has been found that only a very small portion of biotin is dissociated from the complex in a solution of 0.2 M ammonium carbonate at 100°C for 15 minutes. Similar results were obtained by the use of other pH buffer solutions of the same ionic strength. In addition, in solutions of low ionic strength the affinity of avidin for biotin is much less than in solutions of high ionic strength. The present paper reports the results from these experiments on the relationship of heat and ionic strength to the formation and dissociation of the avidin-biotin complex. Similar conclusions with respect to the stability of the avidin-biotin complex to heat and the protective effect of ions on the stability of the complex recently have been reported by Pai and Lichstein(7).

Materials and methods. D-Biotin-carboxyl-C^{14} with a specific activity of 22.9 mc/millimole was kindly provided by Dr. O. Wiss, Hoffmann-La Roche, Basel, Switzerland.

Avidin was purchased from Nutritional Biochemicals Co., Cleveland, Ohio, with an indicated activity of 2.5 units/mg. Pure avidin also was prepared by a new method of Melamed and Green (M. & G.)(8) with an activity of 13.1 units/mg.

The experimental procedures involved in separation of avidin-biotin from biotin by gel-filtration chromatography on a Sephadex G-25 column have been described(6). The dimensions of the column used were 0.8 cm × 33 cm.

The heat stability experiments involved both a study of avidin stability prior to com-

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