THE INFLUENCE OF CORTISONE ON EXPERIMENTAL VIRAL INFECTION

VII. Kinetics of Interferon Formation and Its Inhibition with Hydrocortisone in Relation to Viral Strain and Virulence*

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As a host-produced protective substance formed in response to laboratory infections with many viruses, interferon has received deserved attention as a possibly important determinant of the outcome of viral infections in nature. Indeed, recent reports have demonstrated the presence of interferon in blood (1), cerebrospinal fluid (2), pharyngeal washings (3), and dermal crusts (4) during the course of infection in man. Evidence is contradictory, however, as to whether the synthesis of interferon occurs simply as a consequence of viral infection that happens to coincide with the termination of viral replication, or whether its formation is the cardinal factor in bringing this termination about. In the intact animal host this question is difficult to resolve, especially since the formation of specific and nonspecific immune substances also occurs coincident with recovery from viral infection.

Viral infection of the chick embryo lacks the simplicity of in vitro infection of cell or tissue culture, but comprises an in vivo system less complex than the infected animal. The advantages of this system have been cited (5) and it lends itself well to the study of the pathogenesis of viral infection divorced from the complicating effects of specific antibody formation or temperature response. Also, in the case of influenza virus infection of the cells lining the allantoic sac, cellular destruction and inflammatory response may be quantitated in parallel with virus and interferon formation in assessing the severity or virulence of infection.

The present paper reports studies of the pathogenesis of infection with three

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different influenza viruses in which the kinetics of interferon formation in relation to other measures of host response were investigated and the influence of hydrocortisone on the different measures of response was noted.

Materials and Methods

The experimental procedures were identical with those described in a previous communication on this study (5) with the following additions.

Viruses.—Mel.—The Melbourne strain of influenza A virus was used as a seed with the following characteristics: EID_{50} $10^{9.66}$/ml, HA $10^{4.5}$/ml (40 hr harvest).

Viral Yield.—Measured as the geometric mean of hemagglutinin titrations (HA) of the allantoic fluids of individual eggs, using 0.5% buffered saline suspensions of human A, B, or O red blood cells.

Measurement of Inflammatory Exudate in Allantoic Fluid.—A turbidimetric method for the determination of allantoic fluid protein (6) was employed with minor changes. Two ml of 10% trichloroacetic acid was added at room temperature to 1.5 ml of allantoic fluid that had been clarified by centrifugation for 5 min at 2,500 RPM and then diluted with 0.5 ml phosphate-buffered saline (pH 7.2). Mixing was effected by inversion of the tube. The optical density was read immediately at 420 mμ on a Spectronic 20 colorimeter.

General Experimental Design.—Ten-day embryos were inoculated intraallantoically with 10^3 EID_{50} of one of the three viral strains Lee, Mel., or PR8. The time of viral inoculation was termed zero hour. For standard purposes of comparison, 0.1 mg of hydrocortisone (generously provided by Dr. George M. Shull of Pfizer), or 0.1 ml distilled water control, was administered by the intraallantoic route at (−) 2 to (+) 2 hr. In some experiments the time or dosage of hormone treatment was varied. Eggs were chilled at 4°C overnight and the allantoic fluids harvested for hemagglutinin and interferon assays at intervals throughout a 5 day incubation period at 35°C. The protocols were so arranged that there were overlapping intervals from experiment to experiment, in order to permit observations at as many time periods as possible.

EXPERIMENTAL RESULTS

I. A Comparison of the Pathogenesis and Course of Infection with Three Influenza Viruses in the Chick Embryo

A. Multiplication of Virus.—The multiplication rate of Lee virus, whether measured as egg-infective virus or as hemagglutinating virus, was slower than that of the A strains (PR8 and Mel.). Throughout the present investigation emphasis has been placed upon measurement of viral hemagglutinin because it constitutes a measure of total (inactivated, incomplete, and infective) virus, all of which may contribute to the pathogenesis of infection. Experiments in which infective virus has been measured prior to the appearance of detectable hemagglutinin have corroborated the relative multiplication rates of the three viruses as determined by hemagglutination (HA) titration. Egg infectivity titers (EID_{50}) of allantoic fluids from eggs harvested 14 hr after infection with 10^3 EID_{50} of Lee, Mel., or PR8, were respectively 10^{-3}, $10^{-6.3}$, and $10^{6.2}$. The incremental curves of hemagglutinating (HA) virus are presented in Fig. 1, together with parallel HA determinations of virus increase in eggs injected with hydrocortisone. Interferon activity of fluids from these groups of eggs is

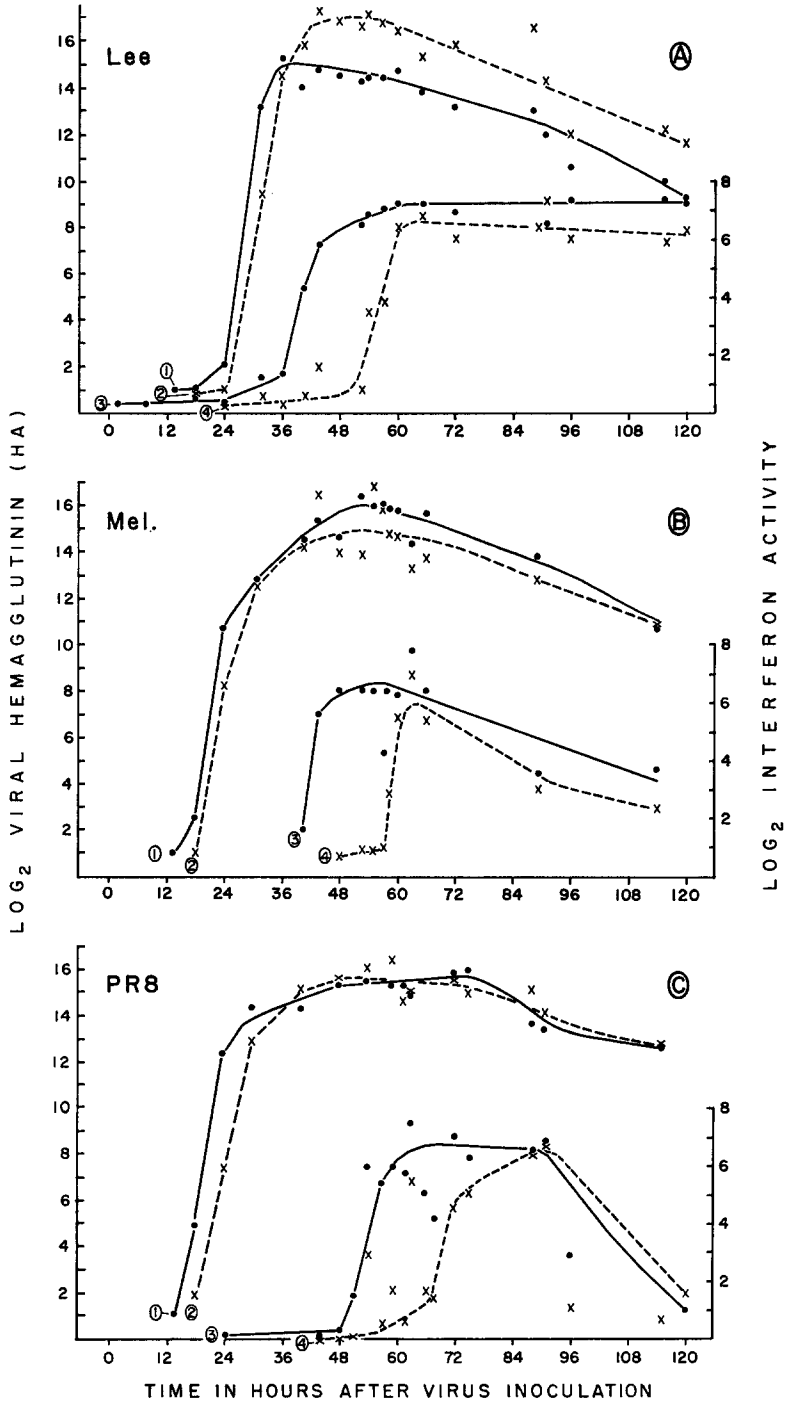

FIG. 1. The temporal relationship of increase in allantoic fluid of viral hemagglutinin (HA) and interferon during infection of chick embryos with three different influenza viruses inoculated in equivalent infective dose (10^8 EID_{50}). Measurements in control eggs are recorded by the solid lines, and in hydrocortisone-injected eggs by the broken lines.

also plotted. It may be seen that Lee virus increases more slowly than either Mel. or PR8, although it attains a comparable maximum titer. The sustained plateau of PR8 is noteworthy and may be related to the relative avirulence of this strain, to be discussed later. Titration of virus in the allantoic sac is obviously a cumulative measure of viral production and does not reflect the level of viral synthesis at any one point in time. However, in the case of PR8, measurement of CAM tissue virus at 18, 44, and 68 hr confirmed the suggestion of a "plateau" of continued viral production. Infectivity titrations of CAM extracts at these intervals were $>10^{-6}$, $10^{-6.3}$, and $10^{-6.5}$ EID_{50}/ml, respectively.

TABLE I

Amount of Interferon Produced by Different Strains of Influenza Virus in the Chick Embryo

Virus inoculated (10^3 EID_{50})	Time of harvest	Inhibitory activity/ml of interferon preparation diluted:			
		Experiment	1:2	1:10	1:20
	hr				
Lee	48	1	5.9*	5.6	3.7
	48	2	6.8	4.8	2.5
	64	3	6.8	6.3	5.7
	72	4	5.5	5.8	5.3
Mel.	48	1	6.8	2.8	1.0
	63	2	7.8	4.5	1.5
PR8	72	1	7.4	4.4	2.4
	91	2	6.7	5.2	1.9
	88	3	6.4	2.4	0.1

* Log_2/ml.

B. Interferon Formation.—It is clear from Fig. 1 that the intervals between the first appearance of hemagglutinating virus and the appearance of detectable interferon differ for the three strains of virus. Interferon activity appeared first in Lee-infected eggs, despite the slower increase of this virus, at approximately 36 hr after infection, while interferon appeared next (at 41 hr) in Mel.-infected eggs and last in PR8-infected eggs some 52 hr after inoculation of virus. Thus the interval between the estimated (interpolated) initial appearance of measurable HA and of interferon in allantoic fluid was only 15 hr for Lee infection, 24 hr for Mel., and 36 hr in the case of PR8. These differing intervals represent real differences in the rates of interferon synthesis because earlier experiments had shown that allantoic fluid interferon activity paralleled closely that of chorioallantoic membrane—the site of viral and interferon synthesis (5). As these earlier experiments had been done principally with Lee

virus, interferon activity was searched for in CAM from PR8-infected eggs during the first 48 hr of infection, but was not found.

Not only the rate of interferon synthesis but also the amount of interferon produced must be considered in any study of the relation of interferon to the course of viral infection. Because in some interferon assay systems a straight line relationship is not found between concentration and activity, interferon is often quantitated (as in the present study) in terms of inhibitory activity (per cent of log reduction of challenge virus) at a single concentration. In Table I the lack of a linear relation between concentration and activity is apparent when the activity of interferon produced in response to the three viruses is assayed at different dilutions. The pertinent point is that interferon from Lee-infected eggs consistently manifested significant viral inhibitory activity at

TABLE II

Susceptibility of Different Strains of Influenza Virus to the Action of Interferon in Vitro

Challenge virus‡	Interferon dilution*			
	Control	1:2	1:10	1:20
Lee B	9.0§	1.0	1.3	4.3
Mel. A	5.3	1.0	2.3	4.0
PR8 A	3.0	1.0	—	—

* Interferon, pH2 dialyzed 72 hr Lee, (−) 20 hr.

‡ Lee B 10^7 EID_{50}, Mel. A $10^{7.5}$ EID_{50}, or PR8 $10^{7.5}$ EID_{50}, 0 hr.

§ 48 hr HA $\log_2$.

higher dilutions than did preparations from eggs infected with Mel. or PR8 viruses.

Another measure of the amount of interferon produced by the viruses is provided by examination of Fig. 1, in which the area beneath the curve (line 3) serves as a measure of the level and persistence of interferon activity during the course of infection. By this criterion, it is clear that infection with Lee virus is attended by a greater cumulative exhibition of interferon than is infection with Mel. or PR8.

Susceptibility of the Three Viruses to the Action of Interferon.—The amount of interferon produced by a given virus is a factor of potential importance in the regulation of infection with that virus. Another important factor in a comparison of viral pathogenicity is the susceptibility of the virus to the action of interferon. This comparison has been made with the three influenza viruses used in the present study, and the results are summarized in Table II. This experiment is compromised by the marked differences among viral yields following inoculation of the three viruses into the CAM culture system in equivalent infective dose. Thus, control values of PR8 HA are just above the level

of detectability (i.e., 1:8 or 3 $\log_2$). Nevertheless, it could be shown that all three viruses were susceptible to interferon action, and a meaningful comparison of Lee and Mel. could be made in terms of $\log_2$ reduction of challenge virus titer at several interferon dilutions.

C. Inflammatory Response. Infection of the allantoic sac of the chick embryo with influenza viruses is attended by necrosis and exfoliation of the entodermal cells and by an increase in allantoic fluid protein measured as trichloroacetic acid-precipitable substances (6, 7). Measurement of this protein increase in comparison to the base line values of uninfected embryos provides a reproducible and useful quantitation of the cytonecrotizing or "inflammatory" effect of influenza viruses that can be used as a measure of viral virulence. This pro-

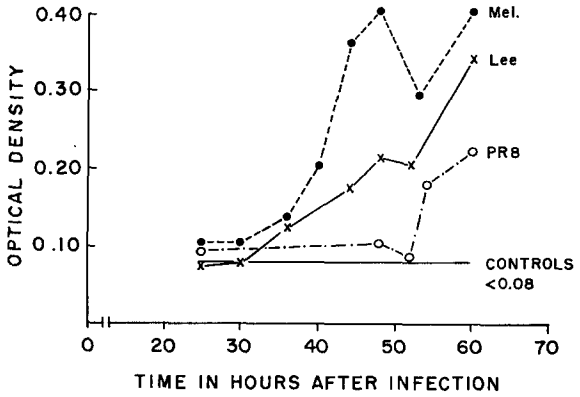

FIG. 2. Inflammatory response to infection of the chick embryo with Lee, Mel., and PR8 influenza viruses as indicated by increase in allantoic fluid protein (measured as optical density [OD] of trichloroacetic acid precipitate).

tein response (7, 8) and other evidences of inflammation (7, 9) are inhibited by antiinflammatory steroids. This technique has been used in the present study as a further measure of the comparative pathogenicity of the three viruses under investigation. In Fig. 2 are charted periodic measurements of optical density (OD) of trichloroacetic acid precipitates of allantoic fluids of groups of embryos (in groups of six) infected with Lee, Mel., or PR8 at equivalent infective dose (i.e., 10^8 EID_{50}). Significant increase in OD above control values (uniformly $<$ 0.08 during the observation period) was first recorded with Lee and Mel. 36 hr after infection but not until 54 hr following infection with PR8. At all subsequent observation periods evidence of greater inflammatory response was apparent with Mel. and Lee.

D. Mortality.—A definitive expression of virulence is death of the host. With the chick embryo the time of death may be fixed by repetitive candling of the egg and determination of the time at which normal blood vessel patterns are

lost and spontaneous movements of the embryos cease (7). Preliminary observations during the course of other experiments indicated that PR8 virus infection under the experimental conditions employed throughout this study was seldom associated with mortality. During the period 5 days after viral inoculation the average mortality rate was only 5%. On the other hand, a

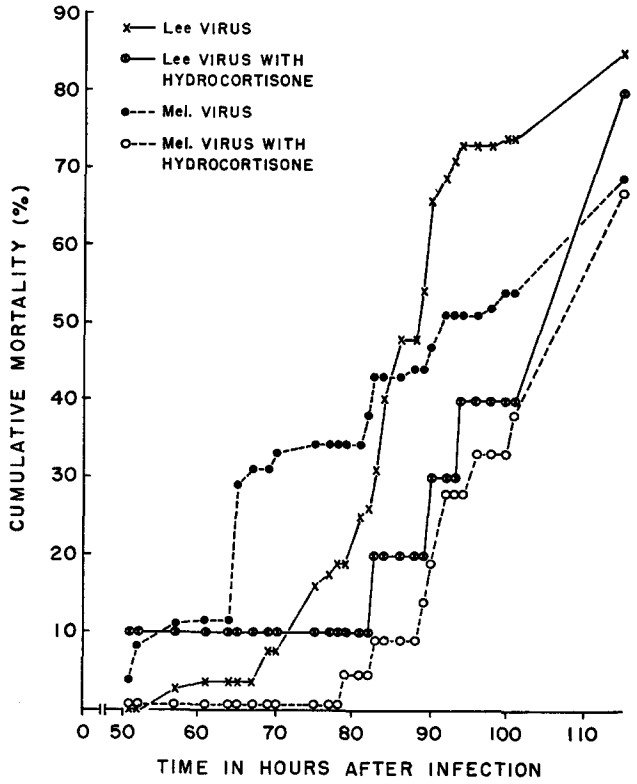

FIG. 3. Cumulative mortality of chick embryos infected with Lee or Mel. viruses showing the influence of hydrocortisone.

progressively increasing mortality rate characterized infection with Lee and Mel. viruses, beginning about 48 hr after infection. In order to compare the relative mortality rates of embryos infected with either Lee or Mel. by an approximation of survival time, an experiment was designed in which 80 embryos were inoculated with 10^3 EID_{50} of Lee and 61 with 10^3 EID_{50} of Mel. virus. Smaller numbers (10 and 21) were injected with 0.1 mg of hydrocortisone 1 hr after inoculation of Lee or Mel., respectively. During the next 115 hr eggs were candled 29 times at intervals averaging approximately 4 hr and the

number of dead embryos was recorded. The results of this experiment are presented in Fig. 3 in which the cumulative mortality associated with infection with each virus is recorded as the per cent of dead/total embryos in each group at each observation time. Although deaths occurred earlier with the Mel. virus, the final mortality rates associated with Lee and Mel. infection were not

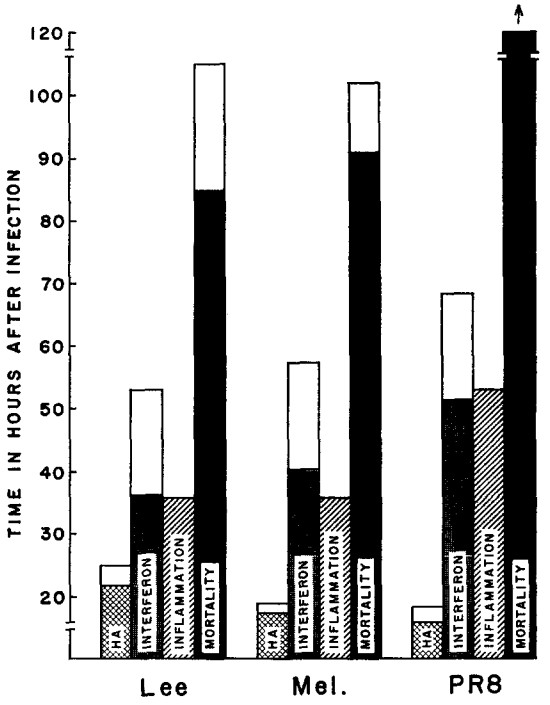

FIG. 4. A comparison of the pathogenesis of infection of the chick embryo with Lee, Mel., and PR8 viruses as indicated by the time of initial detection of viral hemagglutinin (HA), interferon, inflammation (allantoic fluid protein increase), and 50% mortality. The white portions of the bars represent the delay effected by administration of hydrocortisone.

materially different. The increased survival time of embryos injected with either virus and given hydrocortisone is also illustrated.

Integration of the Biologic Attributes of the Three Viruses into a Picture of Viral Virulence for the Chick Embryo.—From the foregoing time sequence studies of the appearance of virus, interferon, inflammatory response, and mortality following infection, a multidimensional diagram may be drawn to define the virulence or pathogenic nature of each of the three viruses under study. Such a diagram is pictured in Fig. 4. In this figure the temporal aspects of infection are summarized in terms of the interpolated times of initial appearance of measurable hemagglutinating virus (HA), allantoic fluid interferon,

increased allantoic fluid protein (inflammation), and 50% cumulative mortality.

On the basis of an earlier appearance of inflammatory response and mortality, the Lee and Mel. viruses may be described as more virulent for the chick embryo host. This despite the earlier appearance of virus and later appearance of interferon in embryos infected with PR8, the virus that must be defined as the least virulent under the present conditions of study.

Several points of interest emerge from this comparison:

The appearance of interferon is not related temporally simply to the attainment of a certain critical concentration of virus, or to the multiplication rate of the virus.

The rate of interferon synthesis is not directly related to viral avirulence or to a favorable outcome of infection.

The time of initial appearance of interferon coincides closely with the beginning of the inflammatory reaction as measured by protein increase in the allantoic sac for each of the three viruses.

The Influence of Hydrocortisone on the Course of Infection with Lee, Mel., and PR8 Viruses

Effect on Virus Multiplication.—As is demonstrated in Figs. 1 and 4, an initial inhibitory effect on virus multiplication was noted with all three viruses in the presence of hydrocortisone. This suppressive effect has been described before for Lee and PR8 (10, 11). The striking augmentative effect on the total and final yields of Lee virus has also been noted previously (12, 10). This effect is not peculiar to Lee virus, but has been noted also with other strains of influenza B, with the Jap. 305 strain of influenza A2 (13), with mumps virus in the chick embryo (12), and with the NWS strain of influenza A in monolayer cultures of human conjunctival cells (13).

Effect on Interferon Formation.—Administration of hydrocortisone 2 hr before to 2 hr after inoculation of virus delayed the formation of interferon by 16 to 18 hr with all three strains. Thereafter the curve of interferon increase and decline roughly paralleled that of control embryos (lines 3 and 4, Fig. 1). For all three strains the period during which hydrocortisone maintained significantly reduced interferon activity in allantoic fluids was also approximately the same, 19 to 22 hr. Yet it is notable that only in the case of PR8 did activity levels in steroid-injected eggs ever reach those of controls.

Although hydrocortisone in a dose of 100 μg/egg inhibited the synthesis of interferon by all three viruses, a titration for the establishment of minimal effective dose (Table III) demonstrated 10- to 1000-fold differences depending of the strain of virus employed. In this experiment, groups of 10-day-old chick embryos were injected with serial decimal dilutions of hydrocortisone ranging from 0.1 to 1000 μg. Interferon activity of allantoic fluids was measured at the

time of peak production characteristic for each virus. Significant reduction in interferon was effected by 1.0 μg/egg in eggs infected with Lee virus, while 10 and 100 μg were required in those infected with Mel. and PR8, respectively. This difference might be attributable to the earlier appearance of interferon in Lee-infected eggs, and hence an earlier opportunity for effects of small and transiently available quantities of steroid on the interferon-forming mechanism; thus, reciprocally the failure of small doses of hydrocortisone to inhibit interferon production in the case of PR8-infected eggs might reflect a longer period for biologic decay of the steroid after injection. On the other hand, if 1.0 μg is injected at −24 hr before inoculation of Lee virus, allowing a comparable

TABLE III

Titration of Minimum Dose of Hydrocortisone Required to Suppress Interferon Formation by Different Strains of Influenza Virus in the Chick Embryo

Hydrocortisone dose	Log_2 interferon activity/ml of allantoic fluid					
	Lee*		Mel.		PR8	
	Control	Hydrocortisone	Control	Hydrocortisone	Control	Hydrocortisone
μg/egg‡						
1000	5.6	1.1	—	—	6.4	1.6
100	5.6	2.4	6.5	0.5	6.4	**1.4**
10	5.1	1.6	6.5	**0.5**	6.4	6.1
1	5.1	**2.4**	6.5	5.0	6.4	6.4
0.1	5.1	4.6	—	—	6.4	6.4

* Time of interferon measurement: Lee 48 hr, Mel. 48 hr, PR8 66 hr, after infection with 10^3 EID_{50} of virus.

‡ At (−) 2 hr.

period for steroid degradation, it is still active in inhibiting interferon synthesis with Lee. The minimum effective dose for inhibition of interferon synthesis during Lee virus infection is identical to that required for significant augmentation of Lee virus yields.

Effect on Inflammatory Response (Protein Increase).—Although earlier studies had demonstrated (7) and more recent studies have confirmed (9, 14) the inhibitory effect of hydrocortisone on virus-induced inflammation in the chick embryo, no such effect was noted under the time-dose relationship of viruses and steroid established in the present experiments. The injection of 0.1 mg of hydrocortisone within 2 hr of virus inoculation had no demonstrable effect on the kinetics of allantotic fluid protein increase as illustrated in Fig. 2. This finding is important, in view of the close temporal coincidence of the initial appearance of increased protein and interferon (Fig. 4), however, and these two sequels of infection are thus separable with hydrocortisone.

Effect on Mortality.—Maximal effects of hydrocortisone on the survival of

chick embryos infected with influenza viruses are brought about by administration of hydrocortisone late in the course of infection at the end of the logarithmic phase of viral multiplication (7). Yet even in the present experiments when hydrocortisone was injected within 2 hr of inoculation of the standard small

TABLE IV

Effect on Viral (HA) Yield and Interferon Formation of Administration of Hydrocortisone at Various Times after Inoculation of Virus

Time of hydrocortisone* injection	HA yield (log_2) difference (Hydrocortisone-control)			Hydrocortisone-associated change in interferon content (log_2) (Control-hydrocortisone)		
	Lee‡	Mel‡	PR8§	Lee‡	Mel‡	PR8§
hr						
−24	+1.0	—	—	−4.5	—	—
−2	+2.2	−0.8	+0.2	−4.4	−5.6	−3.5
2	+2.4	+0.1	—	−4.5	−6.0	—
6	+2.6	—	—	−4.4	—	—
14	+1.2	—	—	−5.1	—	—
16	+1.9	—	—	—	—	—
18	+2.1	—	—	−5.3	—	—
19	+1.4	—	—	—	—	—
20	**+1.5**	(−)1.1	—	−4.5	−4.0	—
21	+0.2	—	—	−3.3	—	—
22	+0.4	—	—	—	—	—
24	−0.1	−0.7	—	**−3.2**	−5.0	—
27	+0.5	−1.5	—	−0.5	**−3.5**	—
30	−2.2	+1.1	−0.9	−0.9	−0.5	−5.0
32	+0.3	—	—	−3.3	—	—
36	−0.5	−2.6	—	−1.0	−1.5	—
40	−0.6	—	—	+0.3	—	—
43	−0.2	+1.1	−1.5	+0.3	−0.3	−4.7
44	—	—	−1.1	—	—	−5.0
48	—	—	−1.8	—	—	−2.2
51	—	—	−2.2	—	—	−1.6
53	—	—	−0.9	—	—	**−2.7**

* 0.1 mg/egg.

‡ Virus and interferon measured at 44 to 48 hr.

§ Virus and interferon measured at 59 to 68 hr.

inoculum of virus, the survival of chick embryos infected with Lee or Mel. was prolonged (Figs. 3 and 4), although the final mortality rate was the same in cortisone and control groups (Fig. 3). (The stepwise progression of the cumulative mortality curves for cortisone-injected embryos in Fig. 3 probably reflects the smaller number of embryos in these groups and the frequent observation periods.)

Although hydrocortisone clearly has other effects in the influenza virus–

infected chick embryo, it is interesting that it may bring about the paradox of diminished and delayed interferon synthesis with prolonged host survival. In a limited number of experiments, the low (5%) mortality rate with PR8 infection was not significantly affected by hydrocortisone during a 6 day observation period.

The Kinetics of Interferon Synthesis during Influenza Virus Infection. Time Factors in Inhibition with Hydrocortisone.—It was of interest to determine whether interferon production could be "shut off" late in infection, and to examine what influence if any this delayed inhibition might have on final yields of virus, especially with those strains (Mel. and PR8) with which augmented yields were not noted with early administration of steroid. In a series of experiments, eggs were inoculated with virus under the usual experimental conditions, except that injection of hydrocortisone was delayed for various periods ranging from 6 to 53 hr after infection. As noted in Table IV, hydrocortisone injected as late as 20 hr after infection was effective in enhancing final yields of Lee virus, while interferon synthesis could be reduced as late as 24 hr afterward. With Mel. and PR8 infection, significant increases in viral yield were not effected by hydrocortisone injection at any time period, yet interferon synthesis was reduced by injection of steroid at 27 hr in the case of Mel., and at 53 hr with PR8. This late susceptibility of interferon synthesis to the effects of hydrocortisone suggests that, in confirmation of the data in Fig. 1, interferon increase continues well beyond the peak of virus multiplication and that its increase in allantoic fluid reflects continued synthesis late in infection rather than merely delayed release from cells. The fact that PR8-infected embryos are influenced latest suggests the continued existence of viable interferon-synthesizing host cells, and indirectly confirms the relative avirulence of the PR8 virus.

DISCUSSION

Two related hypotheses, attractive in their simplicity, would implicate interferon in endogenous termination of viral infection (15–17) and in the avirulence of certain viruses (18, 19). While the present studies refute neither hypothesis, they certainly provide no categorical endorsement for them in this investigation of closely related viruses in an in vivo system. To begin with, the two viruses judged most virulent by the criteria of lethal effect and early induction of inflammatory response (Lee and Mel.) were the earliest inducers of interferon synthesis. In the case of infection with Lee virus the interferon response was also greater and more sustained than with either Mel. or PR8 infection, and had its onset earlier with respect to the initial appearance of hemagglutinating virus. Of perhaps equal importance is the apparently greater susceptibility of Lee to the action of interferon, although comparison of all three viruses in this regard suffers from the lack of an adequate assay system for a PR8 challenge.

While the correlation of viral avirulence and susceptibility to interferon (or increased ability to induce its formation) is striking in monolayer cultures of relatively homogeneous cells (18–21), exceptions are noted in more complex systems. Newcastle disease virus (NDV), lethal for the chick embryo, nevertheless induces formation of interferon in that host (22) and in fact high titers of interferon are first evident at the time of death (19). In primary (heterogeneous) cell cultures of dog kidney, Sellers and Fitzpatrick (23) described the concomitant production of cytopathic effect and interferon production with an influenza B virus but noted an insensitivity of the virus to interferon action.

In the intact animal infected with virulent and lethal viruses, abundant interferon formation may occur in apparent direct relation to the level of virus produced, as in the case of Sindbis virus infection of infant and adult mice. In this situation, markedly higher titers of interferon (and virus) are demonstrable in the brains of lethally infected infant mice than in the brains of adult animals that recover (24). Thus, the present studies are not anomalous, but add further evidence of the lack of a necessary inverse correlation of interferon production and viral virulence, particularly in in vivo systems.

In this connection, the present studies also indicate that the introduction of an exogeneous factor, i.e. hydrocortisone, may complicate further the host–virus–interferon relationship. Thus, hydrocortisone may inhibit transiently the production of interferon out of proportion to its initial suppressive effect on viral synthesis, and yet the survival of the infected host (in which more virus may be produced, in the case of Lee) is prolonged. Hydrocortisone with its multipotential hormonal effects is not as sharp-edged a tool for dissecting out the contribution of interferon to recovery as is actinomycin D (25, 26), for example. On the other hand, it is a naturally occurring substance endogenously present in the intact animal and its potential effects must be reckoned with. In any case, the present instances of prolongation of survival coincident with reduction in interferon synthesis reemphasize that which should be obvious, that interferon is not the single determinant of recovery from viral infection.

The mechanism by which hydrocortisone increases survival time is not clear. Analysis of the apparent "antitoxic" or cell-preserving effect of corticosteroids is complicated even in in vitro systems by their initial suppressive effect on the multiplication of certain viruses (27, 13). Thus, delay in CPE may be only a consequence of delay in the attainment of the critical viral mass associated with cytonecrosis. Even so, most studies in vitro have not found evidence of corticosteroid protection of target cells (27, 13) although delay in the onset of poliovirus (28) and yellow fever virus (29) CPE has been reported, but without concurrent studies of virus multiplication. In the chick embryo, late administration of hydrocortisone is most effective in increasing embryo survival time and reducing inflammation. Therefore, it is unlikely that virus-synthesizing cells are preserved (yields are not augmented beyond 20 hr) but rather the secondary reaction to the destruction of chorioallantoic membrane target cells is reduced

(7, 9). It is notable that final mortality rate is not lessened but rather death is postponed. If hydrocortisone is given early in infection, then its suppressive effect on interferon synthesis (and augmenting effect on viral yield) may predominate, so that survival is only slightly prolonged, as in the present study, or indeed is shortened when a larger virus inoculum is used (7, 9).

These observations have probable relevance to problems involving the use of cortisone in infectious diseases. In experimental infection of *Macaca mulatta* with the virus of Venezuelan equine encephalomyelitis, it was found that treatment with cortisone *after* virus inoculation suppressed the inflammatory response in the central nervous system, while prior injection of cortisone had no such effect, and in fact prolonged the stage of viremia (30). In natural infections of man, it is a fair generalization that the adverse effects of corticosteroids usually occur when the use of hormones precedes the acquisition of infection, and that the short-term use of steroids during the course of a primary acute infection either has no effect or perhaps may be beneficial (31). A striking example is varicella, a usually mild infection that may have a fatal outcome in the child on prior corticosteroid administration (32), yet which may be benefited by corticosteroid therapy for its pneumonic complications in the adult (33, 34).

The puzzling question of why yields of Lee (influenza B) virus but not yields of the influenza A strains Mel. and PR8 are augmented with hydrocortisone is not definitively answered by the present study. Certainly, production of interferon appears to have been comparably diminished with all three strains. However, the time of appearance of interferon in relation to the appearance of detectable HA was earlier with Lee, more interferon was produced, and Lee virus was more susceptible to the effects of interferon than was Mel. Although a satisfactory answer with respect to in vitro interferon susceptibility is not available for PR8, it is clear that in contrast to results obtained with the WS influenza A strain (17) high concentrations of PR8 virus are demonstrable in the CAM as late as 14 hr after the appearance of interferon. Thus, the increase of Lee virus is potentially more subject to regulation by interferon, and conversely, the elimination of this restraining effect (as by hydrocortisone) might be expected to increase eventual viral yields to a greater degree, especially as less hydrocortisone is required to inhibit interferon production with Lee. It should be pointed out that yields of PR8 are slightly but significantly enhanced with higher multiplicity inocula and when measured by more precise methods (12), and that marked augmentation of yields with cortisone attends the inoculation of partially inactivated PR8 virus (10). The greater capacity of inactivated influenza viruses to induce interferon formation has been described (35). The fact that Lee has a shorter half-life at 35°C (85 min) than PR8 (147 min) (36), suggests that in ovo inactivation during infection may contribute to it greater effectiveness as an interferon inducer and reciprocally to its greater accessibility to the influence of hydrocortisone.

SUMMARY AND CONCLUSIONS

A comparative study was undertaken of the pathogenesis of infection of the allantoic sac of the chick embryo with three influenza viruses of differing virulence, and of the influence of hydrocortisone on the course of infection.

Judged on the basis of earlier onset and greater degree of inflammatory response and diminished survival time of infected embryos, Mel. and Lee viruses were markedly more virulent than PR8, despite the earlier appearance of virus in PR8-infected embryos. Interferon appeared first and in greater quantity in the allantoic fluid of Lee-infected embryos and latest with PR8 infection. Thus, there was no correlation of avirulence and better interferon production with the viruses under study in the present system. Furthermore, evidence obtained suggested that Lee virus ("virulent") was most susceptible to interferon action, and also that viral synthesis in the chorioallantoic membrane with PR8 ("avirulent") persisted after the appearance of interferon.

The injection of hydrocortisone within 2 hr of the initiation of infection delayed the synthesis of all three viruses; had no significant effect upon the inflammatory response; and transiently inhibited the synthesis of interferon, while prolonging the survival of Lee- and Mel.-infected embryos. Late administration of hydrocortisone suppresses both the inflammatory response and the production of interferon.

Only in the case of Lee virus infection did hydrocortisone administration lead to augmentation of final yields of virus with the low infection multiplicity employed in the present experiments. It is postulated that Lee virus is a better inducer of interferon because its infectivity in vivo is more rapidly inactivated. As a consequence synthesis of Lee virus is more under the control of endogenous interferon than is the case with PR8 or Mel. virus. Therefore, inhibition of interferon synthesis with hydrocortisone has a greater influence on final yields of Lee virus.

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