# REACTIVATION OF NON-INFECTIVE VIRUS IN A CORTISONE-INJECTED HOST*

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Previous reports from this laboratory have described various effects of cortisone and structurally related steroids on experimental viral infection. Evidence has been presented that: (*a*) final yields of influenza viruses are increased in cortisone-injected chick embryos (1) and mice (2); (*b*) lethal infection with Coxsackie viruses may be induced with cortisone in adult mice (3) and such infection may be associated with extensive lesions of the myocardium, a site not otherwise involved (4).

Investigation of the mechanisms by which cortisone induces effects on influenza B virus reproduction has revealed that: (*a*) the influence of cortisone on influenza virus increase may be mediated at the tissue level (5) remote from possible corollary effects of the steroid on antibody or extrinsic phagocytes, and that, (*b*) the paradoxical effects of prolonged survival and increased virus in cortisone-injected eggs (6) are clearly separable by appropriate timing of cortisone administration (7). Thus, early injection of cortisone results in predicitably increased yields of virus, while delay of injection until the peak of viral increase fails to increase viral concentrations further, and significantly prolongs embryo survival. This therapeutic effect has been correlated with demonstrable suppression of inflammatory reaction in cortisone-injected embryos (7).

Further studies, as yet unpublished, have shown that increased final concentrations of influenza B virus are attained in eggs inoculated either with fully active or partially active viral preparations. The *rate* of viral increase is accelerated with cortisone in eggs injected with sufficient active virus to insure initial infection of all chorioallantoic cells and a "one step" growth curve. It also was observed that cortisone induced relatively high viral yields in eggs injected with inocula which contained large amounts of inactive or non-infective virus. This result suggested a "negation" or cancellation by cortisone of viral autointerference. An independent study employing PR8 virus has demonstrated

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increased yields of hemagglutinin in the presence of cortisone in eggs injected with interfering and challenge viruses (8).

The present communication will demonstrate that homologous or heterologous interference with inactive influenza B virus is apparently negated in cortisone-injected embryos, but that this apparent negation of viral inhibition results rather from the reactivation of non-infective virus in the interfering preparation.

## *Materials and Methods*

*Viruses.*—The Lee strain of influenza B virus and the PR8 strain of influenza A virus were used as allantoic fluid suspensions. Dilutions of virus were made in 0.01 M phosphate-buffered NaCl (0.85 per cent) solution. All viral inocula contained streptomycin (5 mg./ml.) and penicillin (50 units/ml.). Preparations designated as *"active" or "infective" virus* were prepared as suggested by Horsfall (9, 10) to contain fully infective virus. Five successive passages of both viruses were effected at dilutions of $10^{-5}$. Eggs inoculated with PR8 virus were harvested after 24 hours incubation; those infected with Lee virus after 30 hours. Fluids uncontaminated by bacteria were frozen in $CO_2$-ethanol and stored in sealed glass ampoules at −65°C. "Inactive" viral preparations containing relatively few or no infective particles were prepared by various methods of thermal inactivation to be described, including prolonged storage of allantoic fluid virus at 22°C. as described by Horsfall (9). *RDE*[1] (receptor-destroying enzyme) represented the filtrate (No. 2 Coors filter) of a 48 hour neopeptone broth culture of the Inaba strain of *Vibrio cholerae*. This preparation in a dilution of 1:10 destroyed chorioallantoic membrane inhibitor when incubated with membrane suspensions for 3½ hours.

*Cortisone.*—Cortisone acetate (Merck[2]) was employed in aqueous suspension. Microcrystalline suspensions capable of passing through a No. 25 needle were prepared with a Ten Broeck glass grinder. No dispersing agents or special vehicles were used. Control injections consisted of sterile distilled water. *Determination of viral hemagglutinin:* In most instances virus was measured by a modification of the precise fractional dilution technique of Horsfall and Tamm (11). The error of this method in the estimation of allantoic fluid pool titers (5 to 6 eggs) is less than 0.6 $\log_2$ (*i.e.*, 0.6 of a tube in a 2-fold dilution series) (7). If titration of individual eggs early in the logarithmic phase of virus multiplication disclosed wide variation in titers, group titers were expressed as the geometric means of individual egg titrations. *Infectivity titrations* utilized 4 eggs/dilution in a 10-fold dilution series. Allantoic fluids were harvested and tested for hemagglutination in 1:4 dilution after 48 to 72 hours of incubation.

*Expression of Viral Concentration as Number of Infective or Inactive Particles.*—An estimation of the number of inactive (hemagglutinating minus infective) particles has been made by extrapolation from results obtained from Horsfall with a precise enumeration procedure (9). On the basis that $10^{6.09}$ Lee virus particles are required to induce visible hemagglutination in standard 2-fold dilution titrations with a final concentration of 0.25 per cent RBC at 4°C., it may be estimated that the log of the hemagglutination titer +6.09 is equivalent to the log of hemagglutinating particles per milliliter (10). Estimation of the number of infective particles was effected by equating 50 per cent infectivity end-points with 0.69 infective particles (10).

## EXPERIMENTAL RESULTS

*Negation of Autointerference in Eggs Injected with Cortisone.*—It was observed that final yields of virus were increased in cortisone-injected eggs even with

[1] RDE, receptor-destroying enzyme.

[2] Generously provided by Merck & Co., Inc. Rahway, New Jersey.

inocula which contained high proportions of inactive or non-infective virus. This observation suggested a cancellation or negation of the autointerference characteristic of such inocula (12). An experiment representative of several designed to test the influence of cortisone on autointerference with Lee virus is presented in Table I.

Groups of 6 eggs were inoculated by the allantoic route with "inactive" virus, fully infective active virus, or equal amounts of both viral preparations. Inactive virus preparations contained 100,000 times as much non-infective as infective virus. 1 hour after inoculation of virus, cortisone (1 mg./egg) or sterile distilled water was injected, also by the allantoic route. This interval was chosen to allow time for viral adsorption and to minimize possible effects of cortisone on unadsorbed virus.

TABLE I

*Apparent Negation of Interference Phenomenon with Cortisone (Autointerference with Lee Virus)*

| 6 eggs | Inocula | | Cortisone 1 mg./egg | Viral yield* (hemagglutinin) | |
|---|---|---|---|---|---|
| | "Inactive" virus‡ | "Active" virus | | Allantoic fluid pools | |
| | Infective particles = $10^{8.12}$<br>Non-infective " = $10^{7.18}$<br>Total..... = $10^{7.19}$ | Infective particles = $10^{7.80}$ | | Titer§ | Log‖ |
| 1 | + | — | — | 16 | $2.0 \times 10^7$ |
| 2 | + | — | + | 768 | $9.3 \times 10^8$ |
| 3 | — | + | — | 80 | $9.8 \times 10^7$ |
| 4 | — | + | + | 640 | $7.7 \times 10^8$ |
| 5 | + | + | — | 48 | $5.9 \times 10^7$ |
| 6 | + | + | + | 768 | $9.3 \times 10^8$ |

* At 50 hours.

‡ Stored at 24°C. for 12 days.

§ Reciprocal of highest dilution inducing hemagglutination.

‖ Reciprocal of highest dilution inducing hemagglutination. +6.09 = estimated log hemagglutinating particles/milliliter.

Following prolonged (12 day) thermal inactivation at room temperature (averaging 24°C.) allantoic fluid preparations of Lee virus were titered for infectivity and hemagglutinin concentration. Such preparations were then employed undiluted as "inactive" virus inocula. Fully infective virus was diluted to provide inocula corresponding to the residual infectivity of "inactive" virus preparations. After injection of these inocula of equivalent infectivity but widely different total virus content, measurements were made of viral yields with and without cortisone at different time periods—usually during the logarithmic phase of viral increase. An experiment representative of more than 10 performed with Lee virus is summarized in Fig. 1.

It is seen in Table I that low yields of viral hemagglutinin were attained in eggs inoculated with inactive virus (groups 1 and 5) while those which received active virus alone yielded fluids of somewhat higher titer (group 3). In contrast,

those groups which received cortisone developed high and closely equivalent concentrations of virus regardless of the nature of the inoculum. This striking effect was interpreted as a negation of the inhibitory or interfering effect of inactive virus on the reproduction of the infective constituents of the inoculum. The stimulating effect of cortisone on the reproduction of active virus was anticipated on the basis of earlier work mentioned previously (1).

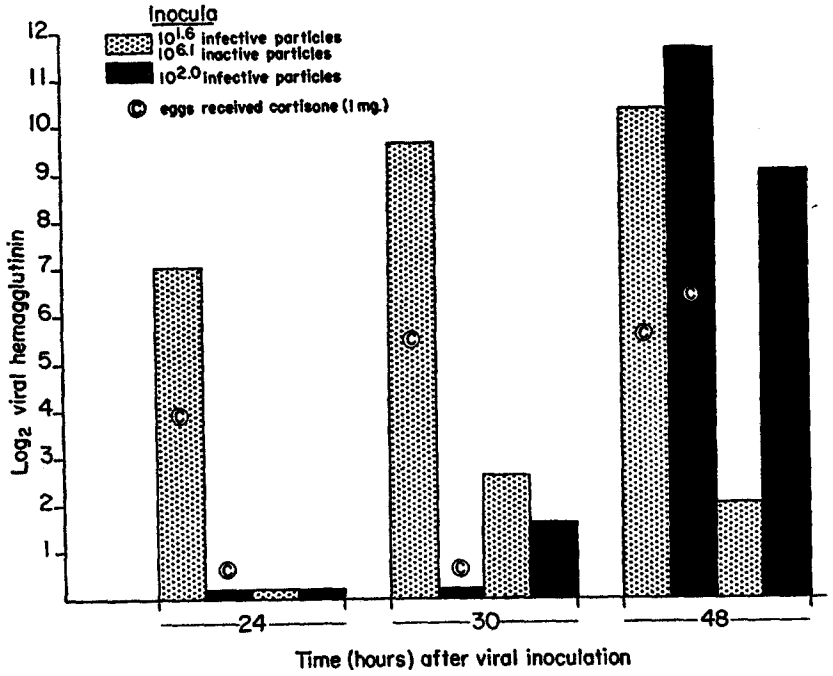


FIG. 1. Accelerated increase of viral hemagglutinin in allantoic fluids of eggs injected with large quantities of inactive (non-infective) Lee virus and cortisone.

*Investigation into the Mechanisms by Which Cortisone Negates Interference. Initial Suggestion of Reactivation of Virus in Cortisone-Injected Eggs.*—The apparent inhibition by cortisone of interference manifest with virus preparations of low infectivity suggested that the infectivity titer of such preparations might be higher in cortisone-injected eggs. As demonstrated in Table II, infectivity end-points with and without cortisone were found not to differ, in accord with past experience with higher titer virus. However, the appearance of the hemagglutination pattern induced by fluids from the $10^{-1}$ dilution in cortisone eggs suggested the presence of greater concentrations of virus in eggs injected with this dilution than were induced with the higher dilutions and

cortisone. Hemagglutination titrations of positive fluids of the infectivity titration confirmed this impression. Not only was the interference evident with the $10^{-1}$ inoculum cancelled by cortisone, but the highest yield of hemagglutinin derived from this lowest dilution in cortisone eggs. The lesser yields of allantoic fluid virus in cortisone eggs at or near the infectivity end-point (dilutions $10^{-2}$ and $10^{-3}$) are explicable on the basis of retention of virus within the chorioallantoic membrane (13) when small inocula are used.

Explanation for the findings of this experiment was sought in the concept

TABLE II

*Initial Suggestion of Viral Reactivation in Cortisone-Injected Eggs: Hemagglutinin Titers of Pooled Allantoic Fluids from Simultaneous Infectivity Titrations in Control and Cortisone-Injected Eggs*

| Dilution of infectivity titration | No. of eggs positive* | | Hemagglutinin titers of positive eggs injected with: | |
|---|---|---|---|---|
| | Control | Cortisone | $H_2O$ | Cortisone |
| $10^{-1}$ | 4/4 | 4/4 | 128‡ | 1536 |
| $10^{-2}$ | 4/4 | 4/4 | 768 | 768 |
| $10^{-3}$ | 2/4 | 2/4 | 1024 | 256 |

* 1:4 dilution of allantoic fluid induces hemagglutination of 0.25 per cent human RBC.

‡ Reciprocal of highest dilution inducing hemagglutination.

of "multiplicity reactivation" described by Luria and Dulbecco with bacterial viruses (14) and subsequently related to influenza virus multiplication by Henle and Liu (15). In brief, it has been shown that when cells are multiply invaded by virus particles rendered non-infective by ultraviolet irradiation, reactivation to a state of infectivity occurs by a mechanism still obscure. Evidence for multiplicity reactivation of influenza virus consists of data indicating that incremental rates with "reactivated" preparations exceed those obtained simultaneously with dilute inocula containing the same concentration of infective virus but little or no inactive virus. Similar evidence is presented below indicating that reactivation of non-infective virus occurs to a striking degree in cortisone-injected eggs.

*Reactivation of Inactive (Non-Infective) Virus with Cortisone.*—In contrast to the marked autointerference evident with the inactive inoculum alone, high titers of hemagglutinin appeared earlier with cortisone and this inoculum than with dilute inocula of similar infectivity with or without cortisone. Growth curves comparable with that shown for cortisone and inactive virus in this experiment have been obtained in this laboratory with inocula containing $10^5$ infective particles (or more than 1,000 times the number of infective particles

per milliliter measured in the inoculum given). It is concluded that cortisone has effected a reactivation of non-infective virus to a state which allows its participation in some manner in the process of viral increase or multiplication.

It appeared possible that the results observed might be explained on the basis of the enzymatic, receptor-destroying potentialities of the large quantity of inactive virus which was injected. This mass of non-infective virus might aid in the early release of newly formed virus from the chorioallantoic membrane into the allantoic fluid, thus spuriously indicating a faster growth rate

TABLE III

*Accelerated Increase of Viral Hemagglutinin in Chorioallantoic Membranes and Allantoic Fluid of Eggs Injected with Cortisone and Inactive Virus*

(Equivalent amounts of infective virus in all inocula)

| Time after inoculation | | Hemagglutinin titers* in eggs injected with: | | | |
|---|---|---|---|---|---|
| | | Cortisone | | $H_2O$ | |
| | | Active virus‡ | Inactive virus§ | Active virus | Inactive virus |
| *hrs.* | | | | | |
| 14 | Membrane | <4 | 800 | — | <4 |
| | Allantoic fluid | <4 | 64 | — | 32 |
| 24 | Membrane | <4 | 3200 | 320 | <4 |
| | Allantoic fluid | <4 | 640 | 8 | 40 |
| 36 | Allantoic fluid | 2048 | 512 | 3072 | 80 |

* Reciprocal of highest dilution inducing hemagglutination (hemagglutinin concentration/unit volume of fluid or membrane).

‡ Active virus—estimated $10^{3.3}$ infective particles injected.

§ Inactive virus—estimated $10^{3.6}$ infective particles and $10^{5.4}$ inactive particles injected.

Note: Titration of saline used to wash membranes revealed residual hemagglutinin only with group injected with inactive virus and cortisone.

than that induced with highly diluted inocula. Direct measurement of membrane and allantoic fluid virus was therefore effected. As indicated in Table III, membrane virus concentration paralleled allantoic fluid virus. Thus, a true accelerated increase in total virus was induced by cortisone with the inactive inoculum. In this experiment as in the preceding one a suggestion of reactivation in the absence of cortisone was observed with inactive virus at the earliest time period. A lag in the increase of highly diluted active virus is also evident with cortisone. Details of this phenomenon will be published elsewhere and are not germane to the present paper.

*The Nature of the Virus Formed Following Reactivation of Inactive Inocula.*—In most experiments no distinction was made between formation of infective

virus and hemagglutinating, but inactive, virus. The possibility that only "incomplete" non-infective virus (16) resulted from reactivation of inactive virus has been excluded on the basis of a few preliminary experiments, although detailed quantitation of the relative yields of infective and inactive virus has not yet been effected. Data indicating a high yield of infective virus following reactivation are presented in Table IV.

*The Nature of Inactive Virus Which Can Be Reactivated with Cortisone (Reactivable Virus).*—Studies by other investigators have established the heterogeneity of influenza virus preparations inactivated by heat (9), or ultraviolet irradiation (15). Depending on the intensity or duration of the inactivating stimulus, properties of infectivity, toxicity (17), interfering capacity (18), and

TABLE IV

*Yields of Infective Virus (Lee) Following Inoculation of Eggs with Inactive Virus and Cortisone*

| Inocula | Cortisone | Viral yield-log infectivity | |
|---|---|---|---|
| | | Hours after inoculation | |
| | | 27 | 36 |
| Inactive virus* | + | 6.0 | 6.7 |
| Inactive virus | — | 4.3 | 2.3 |
| Active virus‡ | + | 4.7 | 7.7 |
| Active virus | — | 4.5 | —§ |

* $10^{0.9}$ infective particles. $10^{7.7}$ inactive particles.

‡ $10^{1.3}$ infective particles.

§ Eggs accidentally discarded.

ability to elute from the RBC substrate (19) are progressively lost during inactivation. Early in the present study it was noted that reactivable preparations of Lee virus also possessed the capacity to interfere with the multiplication of active virus. As was previously described, such preparations had been subjected to relatively mild thermal inactivation at 24°C. Attempts to induce reactivation of virus heated for brief periods (20 to 60 minutes) at 56°C. proved fruitless. The inadequacy of such virus as an interfering agent has been emphasized recently (9) and noted repeatedly in this laboratory. Thus, preliminary evidence equated reactivability with interfering capacity. In a search for reactivable virus totally devoid of infectivity, a preparation was fortuitously discovered which interfered markedly with virus multiplication, but could not be reactivated in the presence of cortisone. This virus had been stored at 4°C. for 66 days. The use of this preparation in experiments to be described permitted for the first time an accurate assessment of the importance of reactivation in negation of interference by cortisone.

The fact that inactivation of Lee virus at 56°C. destroys its enzymatic

function (eluting capacity) (19) as well as its reactivability with cortisone suggested a correlation of the two properties. Adsorption-elution curves illustrative of the enzymatic activity of several viral preparations are presented in Fig. 2.

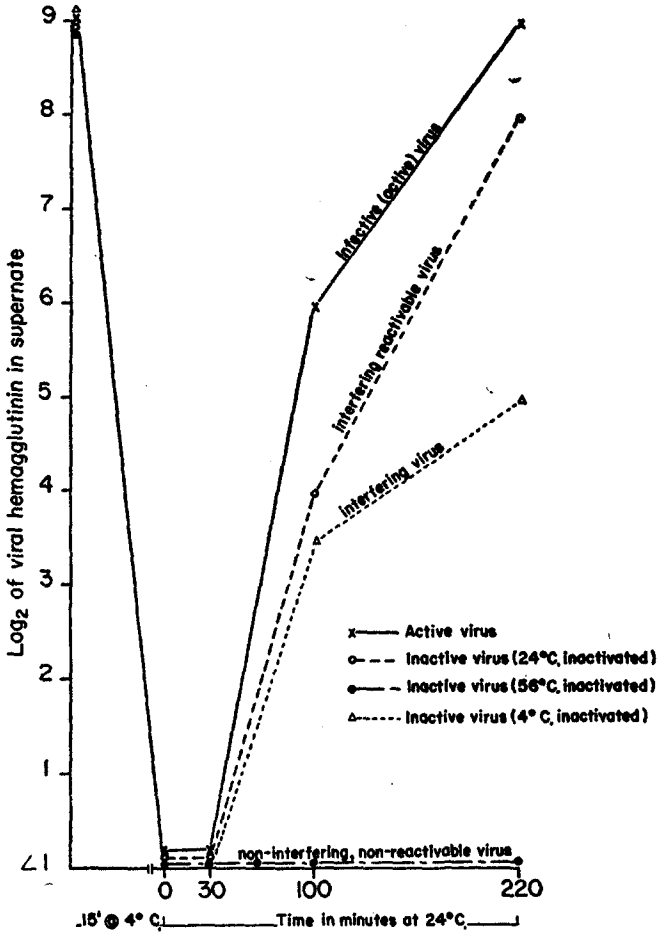


FIG. 2. Relation of the enzymatic activity (elution from RBC) of several inactive virus preparations to their interfering and reactivable properties.

In the experiment shown, equal amounts of hemagglutinating virus were added to a 2 per cent chilled suspension of human O RBC, then held at 4°C. for 15 minutes. Elution was effected at 24°C. Supernates of low speed centrifugations were removed at the times shown and tested for hemagglutinating activity.

After 220 minutes, 50 per cent of the reactivable preparation (inactivated at 24°C.) had eluted from the red cell substrate—a proportion of virus far in

excess of the negligible quantity of infective virus present ($5 \times 10^{-7}$ per cent of total virus). A smaller proportion of interfering but non-reactivable virus had eluted at the termination of the experiment, while the 56°C. preparation which exhibited neither interfering nor reactivable properties failed to show enzymatic activity.

Whether or not the correlation of biologic and enzymatic activity has meaning in terms of influenza virus multiplication, it is useful at present in the classification of inactive Lee virus preparations. As a guide to future studies, a thermal inactivation gradient of viral activity is tentatively proposed in Table V.

TABLE V

*Thermal Inactivation Gradient of Viral Activity (Lee Virus)*

| Property | Types* of viral particles | | | |
|---|---|---|---|---|
| | "Active" | "Inactive" | | |
| | 1 | 2 | 3 | 4 |
| Infective | + | 0 | 0 | 0 |
| Reactivable with cortisone | + | + | 0 | 0 |
| Interfering | + | + | + | 0 |
| Eluting (enzymatically active) | + | + | ± | 0 |
| Hemagglutinating | + | + | + | + |

* 1, fully infective virus; 2, virus inactivated at 24°C. for 12 days; 3, virus inactivated at 4°C. for 66 days; 4, virus inactivated at 56°C. for 45 minutes.

It is evident that the methods of thermal degradation thus far employed have been unsystematic. Systematic studies of this problem are in progress to effect sharper definition of the energy (heat) required for inactivation of the several properties of influenza virus.

*Reinvestigation of "Negation of Interference" Induced by Cortisone.*—In early experiments it was impossible to determine whether the cancellation of interference with cortisone was solely the result of reactivation of the inactive interfering virus. Discovery of a preparation which was interfering but non-reactivable prompted reinvestigation of this question.

Groups of 6 eggs were inoculated with sterile saline or with an undiluted, non-infective Lee virus preparation containing an estimated $10^{8.1}$ particles. 2 hours later a $10^{-3}$ dilution of fully infective PR8 virus ($10^{8.2}$ particles/egg) was injected into all eggs. 1 hour after the inoculation of PR8, cortisone was injected into half the eggs singly or doubly infected.

In Fig. 3 the results are shown of hemagglutinin assays at 17 and 21 hours after injection of the active virus. It is evident that in both cortisone and control eggs interference with the growth of PR8 has been effected. Thus heter-

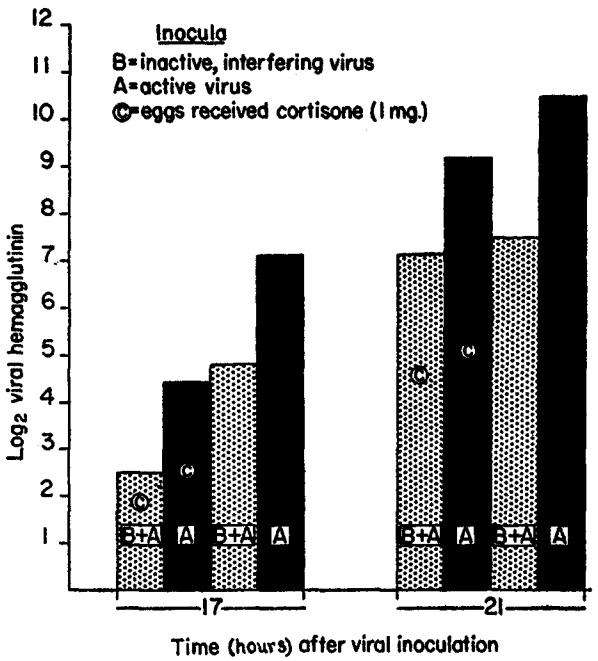


FIG. 3. Failure of cortisone to negate heterologous interference of inactive Lee with active PR8 virus in the absence of reactivation of the interfering virus. Hemagglutinin in all groups was serologically identified as influenza A.

TABLE VI

*Failure of Cortisone to Negate Autointerference with Lee Virus in the Absence of Reactivable Virus*

| Time after injection | Inactive virus* (0 hrs.) | Active virus‡ (2 hrs.) | Cortisone (3 hrs.) | Yield of viral hemagglutinin§ |
|---|---|---|---|---|
| *hrs.* | | | | |
| 23 | + | + | + | 6.1 |
| 23 | 0 | + | + | 16.0 |
| 31 | + | + | + | 182.0 |
| 31 | 0 | + | + | 2200.0 |

* Estimated $10^{8.1}$ inactive particles; <1 infective particle.

‡ Estimated $10^{4.6}$ infective particles injected/egg.

§ Reciprocal of highest dilution of virus inducing hemagglutination; geometric means of fluids from 5 eggs.

ologous interference of Lee with PR8 may occur in the cortisone-injected host. Evidence that autointerference of inactive Lee virus may also be uninfluenced by cortisone is presented in Table VI. The available evidence thus suggests that negation of interference with Lee virus is only apparent, and does not occur in the absence of reactivation of the interfering virus.

*Is Reactivation Dependent upon Multiplicity?*—Thus far, all "inactive" preparations of virus which were reactivable have contained small amounts of infective virus. Dilution sufficient to free inocula of infective virus has of necessity diluted the inactive particles to a point beyond the number ($>1.8 \times 10^7$) necessary to allow multiple infection of chorioallantoic membrane cells (9). It is thus still unclear whether traces of infective virus or multiple invasion of

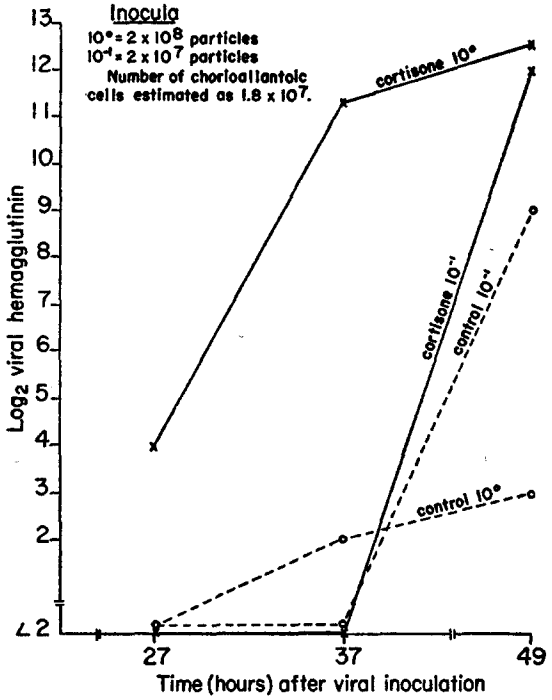


FIG. 4. Evidence for the importance of multiplicity in cortisone-induced reactivation of Lee virus. Radically differing growth curves resulting from dilution of inactive virus sufficient to prevent multiple invasion of host cells.

cells by inactive virus—or both—are the *sine qua non* of cortisone-induced reactivation. However, the comparative viral incremental curves shown in Fig. 4 stress the importance of a high particle-cell ratio in effecting rapid rates of hemagglutinin formation with inactive virus and cortisone. The high final yields of virus resulting from inoculation of the $10^{-1}$ dilution (with or without cortisone) are explicable on the basis of reduction of the particle-cell ratio so that autointerference has been lessened. The greater yield effected with cortisone in this instance may be explained as a consequence of its effect on the residual infective virus of the $10^{-1}$ inoculum.

*The Site of Action of Cortisone in Viral Reactivation.*—The fact that cortisone

may induce its effects on influenza B virus multiplication by either the allantoic or yolk sac route of injection is evidence that the action of cortisone is on the cell or the cell-virus complex, rather than on the virus particle itself. Prior incubation of inactive virus with cortisone for 24 hours does not induce reactivation if virus is separated from cortisone before its inoculation.

## DISCUSSION

It is almost tautologism to state that a virus is intimately and definitively dependent upon the host cell for its multiplication, and hence for the definition of its ultimate property of "infectivity." This dependence of the parasite upon the host environment can receive no stronger emphasis than is provided by the present study; evidence is adduced that the administration of a chemically defined agent (cortisone) may so alter the *milieu interieur* of host cells that virus defined as "inactive" in pristine cells is enabled to participate in the reproductive process.

Present evidence suggests that the reactivation effected with cortisone is dependent upon inoculation of sufficient virus to insure a high proportion of virus particles to host cells, and probably, therefore, upon the presence within each cell of more than one inactive particle (multiplicity reactivation). Thus, it is likely that cortisone-induced viral reactivation is not a qualitatively new phenomenon totally without precedent. The evidence of Henle and Liu that multiplicity reactivation might occur with influenza viruses (15) has received support in certain experiments of the present study in which inoculation of inactive virus without cortisone induced the accelerated appearance of hemagglutinin despite extensive autointerference. It is notable, however, that the magnitude of reactivation achieved with cortisone so far exceeded that previously described, that an apparently new phenomenon—"negation of interference"—was observed with inocula which contained little infective virus. This is in contrast to the observation of Henle and Liu that no detectable levels of hemagglutinin appeared with preparations in which inactivation had induced a great decrease in infectivity. Although most experiments performed by the aforementioned investigators used ultraviolet inactivation procedures, similar effects were demonstrated with heat-inactivated virus.

It is of interest that the chemical inhibition of influenza virus increase by DRB,[3] an *N*-glycoside of benzimidazole, (20) is significantly diminished in cortisone-injected eggs (13). If it be assumed that the capacity of a cell to support viral multiplication is a sensitive indicator of the metabolic integrity of the cell, then present evidence suggests the diffuse but convenient concept that cortisone aids in the maintenance of cellular metabolic integrity during the insult of viral infection. This protective effect (7), which is demonstrably capable of prolonging survival of infected embryos, paradoxically results in

[3] DRB, 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole, courtesy of Dr. Karl Folkers, Merck & Co., Inc.

increased yields of infecting virus in the face of assaults on viral synthetic mechanisms by a chemical agent (20) or by the toxicity of the virus itself. If this hypothesis is applied to the phenomenon of reactivation, it must be assumed that "non-infective" virus ordinarily induces sufficient cellular damage to prevent its own replication—which might otherwise occur in an optimally functioning cell (*i.e.*, with cortisone). Although influenza virus toxicity is closely associated with infectivity, toxic effects in chick embryos have been described with non-infective, but interfering virus (21). A further assumption must be made that the demands on the host viral synthetic mechanisms are relatively greater when degraded virus is introduced than when fully infective virus is injected, and that the injurious effect of fully infective virus is relatively less than that of inactive but reactivable virus. Reactivation of ultraviolet-inactivated bacteriophage adsorbed to host cells may be effected by the addition of extrinsic energy to the host-virus system in the form of visible light (photoreactivation) (22). It is possible that the function of cortisone in influencing influenza virus reactivation and multiplication is related to the endogenous preservation of high energy sources (oxidative phosphorylation) which are of demonstrable importance in influenza virus multiplication (23, 24).

Another hypothesis may be tendered concerning the striking influence of cortisone on viral synthesis. The demonstration by Kun in this laboratory of the occurrence of hexose-6-phosphate dehydrogenase in the chick embryo chorioallantoic membrane has indicated the existence of alternative pathways of glucose metabolism in this tissue (25). The dependence of rat hexose-6-phosphate levels on cortisone has been shown by Conway and Hingerty (26). It is conceivable that cortisone may "unlock" an alternative or additional pathway of carbohydrate metabolism in the chick embryo which is incidentally more effective in the synthesis of virus and permits the utilization of less differentiated, "inactive" virus in the multiplication process. This possibility is under study.

## SUMMARY

The administration of cortisone to chick embryos inoculated with large quantities of inactive influenza B virus results in a rate of viral increase greater than is concomitantly observed with inocula of comparable infectivity which are devoid of inactive particles. Thus, more than a mere negation of autointerference is effected.

It is concluded that in the presence of cortisone reactivation has occurred of non-infective virus to a state in which it can participate in viral synthesis.

Cortisone-induced viral reactivation is dependent upon a high particle/cell ratio and is thus analogous to the previously described phenomenon of "multiplicity reactivation."

Cortisone does not influence either homologous or heterologous viral interference unless reactivation of the inactive interfering virus occurs.

Virus reactivable with cortisone possesses both interfering and enzymatic properties.

Reactivation of virus with cortisone cannot be effected *in vitro* but is mediated by the host cell.

Two hypotheses concerning the action of cortisone are presented.

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