PRODUCTION OF AN INTERFERON BY L CELLS INFECTED WITH WESTERN EQUINE ENCEPHALOMYELITIS VIRUS

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ABSTRACT

LOCKART, ROYCE Z., JR. (The University of Texas, Austin). Production of an interferon by L cells infected with Western equine encephalomyelitis virus. *J. Bacteriol.* **85**:556-566. 1963.—Two strains of Western equine encephalomyelitis virus (WEE), WEE (L+) and WEE (L-), which differed with respect to their cytopathogenicity for L cells were isolated. Both strains reproduced in L cells, and both induced the production of an interferon distinct from virus particles. L-cell monolayers were protected from degeneration by prior addition of interferon. By use of the absence of cytopathic effects (CPE) as an end point, interferon content was assayed. Monolayers failing to show CPE consistently produced less than 2% as much virus as control monolayers, indicating that virus synthesis was also inhibited. The use of this assay method was facilitated by the use of horse serum that appeared to contain antibodies against WEE and that permitted interferon to act selectively in the presence of active virus. It was found that interferon was produced during the time in which active virus was produced, and not significantly later. No interferon could be found in fluids from cells treated with inactive virus, although these are known to act as interfering agents. Interferon production was inhibited by pretreatment of L cells with sufficient amounts of interferon. It is concluded that interferon production is closely connected with WEE virus synthesis in L cells. The question is raised as to whether interferon need be a necessary intermediate for interference in L cells.

Chambers (1957) described a carrier state that frequently resulted from the infection of L cells with Western equine encephalomyelitis virus (WEE). She proposed that autointerference was responsible for the persistent infection observed. Lockart (1960), working with

the same virus-host system, showed that homologous interference by inactive WEE also frequently led to the establishment of the carrier state. Virus persistence was postulated to be the result of a dynamic equilibrium between a state of transient cellular resistance due to interference and virus production by susceptible cells.

Isaacs and Lindenmann (1957) found that an interfering material, which they called interferon, was produced by cells treated with inactive influenza virus. Henle et al. (1959) and Ho and Enders (1959) found that interfering materials separable from intact virus particles and similar to interferon existed in carrier cultures, and reported these to be responsible for the carrier state.

There has been a scarcity of reports on the production and study of interferons in continuous-cell lines (Ho, 1962).

The present work demonstrates that L cells infected with WEE produced an interferon. Interferon production and interferon action in the homologous system are described.

MATERIALS AND METHODS

Tissue culture. L cells (strain 929) were obtained from Norman P. Salzman, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. Cells from a single isolated clone were selected and propagated to provide the stock cultures used throughout. Cells were grown on the surface of glass containers in Eagle's (1955) medium supplemented with 5% horse serum. Eagle's medium was used for all experiments, either without serum or with 5% horse (HS-5) or calf serum (CS-5).

Chick-embryo monolayers were prepared according to the method of Dulbecco and Vogt (1954) and grown in Eagle's medium containing 3% calf serum. Monolayers were prepared in 100-mm petri dishes.

Virus. The Western equine encephalomyelitis

virus strain originally used was described previously (Lockart and Groman, 1958). Upon reisolation from mouse brain, two plaque-size mutants on chick-embryo monolayers were noted. Each was purified by two successive single-plaque isolations. On chick-embryo monolayers, one strain formed plaques 2 to 3 mm in diameter, whereas the other produced plaques 8 to 15 mm in diameter when stained 48 or 72 hr after plating. Both were neutralized by specific antiserum prepared against WEE. Neither type formed plaques on L cells. The addition of from 10 to 50 small-plaque-forming units (PFU) per cell to L-cell monolayers resulted in the destruction of all cells by 72 hr after infection. This strain was designated WEE (L+). The large-plaque-forming variant was designated WEE (L-). It was able to multiply on L cells but caused no detectable cytopathic effects (CPE). Stock suspensions of both virus strains were dispensed in 1-ml quantities into stoppered tubes and kept at -70°C until used.

Virus assay. The titration procedure for infective virus was previously described (Lockart and Groman, 1958). The only change was in the overlay medium, which consisted of Eagle's medium with 1% calf serum. Cell monolayers were stained with neutral red 48 hr after infection, and plaques were counted after a few hours of incubation. As a result of the decreased amount of calf serum, an increased efficiency of plating and the formation of larger plaques were obtained. Titers were determined from the average number of plaques on duplicate chick monolayers.

Assay of interfering ability. Variations will be described in the text, as employed. In general, L cells were dispersed with trypsin, and replicate inocula containing 4×10^5 cells in 5 ml of medium were dispensed into 60-mm petri dishes. These were incubated for 48 hr at 37°C in a humidified atmosphere of CO_2 and air. The medium was removed, and 2.0 ml of the appropriate dilution of interferon in growth medium were added to duplicate monolayers. After incubation overnight, inocula were removed, and the monolayers were washed two times with phosphate-buffered saline (Dulbecco and Vogt, 1954) which contained 0.1% bovine serum albumin fraction V (Mann Research Laboratories, New York, N.Y.). This solution was termed PBS + BSA. Cells were challenged by adding

1.5 ml of WEE (L+) diluted in PBS + BSA to a concentration of 2×10^7 PFU per ml or greater. Adsorption was carried out for 1 hr at room temperature. Inocula were removed, and 5 ml of Eagle's medium HS-5 were added. Challenged untreated, and normal unchallenged controls were included with every test. When measurements of virus production were to be made, the cells were washed three times with PBS + BSA prior to feeding. Cell monolayers were observed under an inverted microscope and scored for degeneration 48 and 72 hr after challenge infection. All assays were carried out in duplicate.

RESULTS

Interference by noncytopathogenic active virus. WEE (L+) virus, inactivated by prolonged incubation at 37°C , was shown (Lockart and Groman, 1958) to be capable of interfering with homologous active WEE (L+). Interference by those virus suspensions was abolished by the addition of specific antiserum or by their prior centrifugation. Thus, inactive virus particles may invoke interference. Experiments were performed to determine whether active noncytopathogenic virus [i.e., WEE (L-)] was also capable of conferring immunity.

L-cell monolayers were washed with PBS + BSA. WEE (L-), prepared on chick-embryo cells, was diluted with PBS + BSA, and 1.5-ml samples were added in duplicate to monolayers. Adsorption was carried out at room temperature for 2 hr. Inocula were removed, and 5.0 ml of Eagle's medium HS-5 added per monolayer. Medium was removed 24 hr later, and the monolayers were challenged with WEE (L+) by the addition of 2.1×10^8 PFU in 1.5 ml of PBS + BSA, as described above. The appearance of CPE as a result of challenge by WEE (L+) was prevented by the addition of about 1×10^8 PFU or more of WEE (L-) 24 hr previously (Table 1). The interfering ability of WEE (L-) suspensions was not reduced after incubation for 1 hr at 37°C with 1.5 mg/ml of purified trypsin (Mann Research Laboratories, New York, N.Y.) or by treatment with sufficient ultraviolet light (2,537 Å) to inactivate 5 to 6 logs of the virus. Interfering ability was, however, abolished by centrifugation, thus indicating further that the interference was due to virus particles.

TABLE 1. *Interference by active Western equine encephalomyelitis (L-) virus*

Expt no.	WEE (L-) added ^a	Immunity to CPE ^b
1	50	Complete
2	36	Complete
1	16	Complete
2	12	Complete
1	5.3	Partial
2	4.0	Partial
1	1.8	None
2	1.3	None
1	0	None
2	0	None

^a Amount added (PFU $\times 10^7$) 24 hr before challenge.

^b Degeneration was determined 48 and 72 hr after challenge with WEE (L+). Immunity to CPE is inversely related to the degree of CPE.

TABLE 2. *Demonstration of an interferon after removal of virus by centrifugation^a*

Dilution added	Material added prior to challenge			
	Inactive virus ^b uncentrifuged	Inactive virus ^b supernatant ^c	WEE (L-) stock ^d uncentrifuged	WEE (L-) stock ^d supernatant
1:3	Complete	None	Complete	Complete
1:9	Partial	None	Complete	Complete
1:27	Partial	None	Partial	Partial
1:81	None	None	None	None

^a Results expressed as immunity of L cells to CPE after challenge. L-cell monolayers were treated overnight with 2.0 ml of the above preparations. They were then washed two times with PBS + BSA and challenged by the addition of 2.4×10^7 PFU of WEE (L+). The amount of degeneration was determined 72 hr later. Immunity to CPE is inversely related to the amount of CPE which resulted.

^b A WEE (L-) stock suspension was prepared on chick cells and inactivated by incubation at 37 C for 144 hr.

^c Centrifuged at $100,000 \times g$ for 90 min at 4 C.

^d A WEE (L-) stock suspension was prepared by harvesting the fluid 23 hr after infecting a culture of L cells. It had a titer of 8×10^6 PFU per ml.

Demonstration of an interferon. To determine whether an interferon separable from virus was present, fluids from L cells infected with either WEE (L+) or WEE (L-) were centrifuged in a 40 rotor of a Spinco preparative centrifuge

(model L) at $100,000 \times g$ for 90 min. The upper two-thirds of the supernatant was carefully removed with a capillary pipette and assayed for interfering activity. In the experiment illustrated in Table 2, fluid removed 23 hr after infection of L cells with WEE (L-) was centrifuged simultaneously with an inactive virus suspension that had been prepared in chick-embryo cells and was capable of interference. No interferon for L cells could be demonstrated in virus suspensions produced in chick-embryo tissues, although they contained an interferon for the homologous cell type. This species specificity agrees with that reported for other interferons (Isaacs and Westwood, 1959; Wagner, 1960). The active and inactive WEE (L-) stock suspensions in the present work, and the WEE (L+) stocks reported on previously (Lockart and Groman, 1958), were prepared in chick cells. It is now clear why they fail to induce interference on L cells after the removal of virus by centrifugation or treatment with WEE antiserum. The PFU titer of the WEE (L-) suspension was 8×10^6 PFU per ml before centrifugation. The uncentrifuged fluid thus did not contain sufficient virus for interference due to virus. After centrifugation, the supernatant fluids from both the WEE (L-) suspension and the inactive virus suspension were assayed for interfering ability. Samples of uncentrifuged WEE (L-) and inactive virus suspensions were also assayed for interfering ability. This assay was carried out in the following way. L-cell monolayers (48 hr) were washed two times with PBS + BSA. Fluids to be assayed were diluted with PBS + BSA, and 1.5 ml of varying dilutions were added to duplicate L-cell monolayers. The monolayers were incubated at 37 C for 4 hr, at which time 2.0 ml of Eagle's medium HS-5 were added to each. The monolayers containing virus and medium were then incubated overnight. The fluid was then removed, and the cells were challenged by the addition of 2.4×10^7 PFU of WEE (L+) in 1.5 ml of PBS + BSA. After 1 hr at room temperature, 5.0 ml of Eagle's medium HS-5 were added to each culture, and they were returned to 37 C.

Centrifugation completely removed the interfering activity from the suspension containing inactive virus but failed to reduce the interfering activity of the WEE (L-) fluid.

The presence of an interferon was also demonstrated in fluids from L-cell cultures containing WEE (L+) after the removal of virus by centrifugation.

These results agree with those of other workers who have demonstrated the existence of interfering factors which can be separated from the virus by ultracentrifugation (Isaacs and Lindenmann, 1957; Ho and Enders, 1959; Cooper and Bellett, 1959).

Properties of the interferon. The interferon described here shares many properties with interfering materials of other systems (Bellett and Cooper, 1959; Chany, 1961; Henderson and Taylor, 1960; Ho and Enders, 1959; Isaacs and Lindenmann, 1957; Wagner, 1961). Tests of certain properties were made on interferon prepared in serum-free medium. These are summarized in Table 3. Fluids were harvested from 24 to 72 hr after infection of L cells with WEE (L-). The interferon produced by WEE-infected L cells appears to be very similar to the other interferons reported.

Correlation of CPE with virus production. A gradation in the amount of CPE was seen, usually within three or four serial twofold dilutions of interferon. The end point for interference was chosen as that dilution of interferon which permitted no visible disruption of the cell monolayers by active virus. There was occasionally, however, some free cell debris in the fluid medium. Those monolayers which received the next twofold dilution of interferon generally showed marked cytopathic changes. The end point was determined 72 hr after challenge, when infected control monolayers were completely degenerated. Figures 1a, 1b, and 1c show representative views of monolayers incubated overnight with 1:32, 1:64, and 1:128 dilutions of interferon, respectively, before challenge with WEE (L+). The monolayers were fixed and stained 72 hr after challenge. The monolayers receiving the 1:64 dilution of interferon were considered to indicate the end point, as adjudged by the absence of CPE. They produced 1.4% the amount of virus produced by the controls. The monolayers which received interferon diluted 1:128 showed marked degeneration, significantly more than reflected by their virus production, which was 9.0% of the controls. The CPE and virus production of

TABLE 3. *Properties of interferon*

Treatment	Effect on interfering ability ^a
Trypsin (5 mg/ml) for 1 hr.....	Destroyed
Ether extraction.....	Destroyed
70 C for 30 min.....	Destroyed
55 C for 30 min.....	Reduced
37 C for 20 hr.....	None
4 C for 8.5 months	None
Centrifugation (4 hr at 40,000 rev/min).....	None
Ultraviolet irradiation for 30 min..	None
Dialysis (17 hr against PBS at 4 C)..	None
Freezing and thawing (5 times)...	None
Ribonuclease for 30 min at 37 C (10 µg/ml).....	None
Deoxyribonuclease for 30 min at 37 C (10 µg/ml).....	None

^a Two interferon suspensions were prepared in serum-free medium. They prevented CPE in L cell monolayers at dilutions greater than 1:10. After the treatment above, each preparation was reassayed for its titer and was termed destroyed if no interference was seen at the 1:2 dilution.

the monolayers depicted in Fig. 1a, 1b, and 1c are tabulated in experiment 3, Table 4.

Table 4 shows three interferon assays. It will be noted that those monolayers showing no degeneration made 2%, or less, the amount of virus produced by controls; thus, the absence of CPE also indicates significant inhibition of virus production. In those monolayers showing partial CPE, numerous instances have been noticed in which the per cent virus yield was significantly less than the amount of CPE which occurred. This might be a result of partial interference in many cells, characterized by a reduced yield of virus, but of insufficient interference for their protection from CPE.

Use of inhibitory horse serum (IHS) for interferon assays. A number of horse sera were found that prevented the infection of L cell monolayers inoculated with WEE (L+). The addition of as many as 5×10^9 PFU of WEE (L+) failed to produce CPE on L cells when added to medium containing 5% IHS. Inhibitory sera did not affect the virus susceptibility of cells. L cells incubated overnight or grown for three generations in IHS-containing medium were fully susceptible after they were washed and infected, and regular medium was added.

FIG. 1a. *L* cells, 72 hr after infection, that had received 2.0 ml of a 1:32 dilution of interferon 16 hr previously. Magnification: $\times 80$.

Virus yields from such cultures were equal to those of cultures of cells grown in medium containing noninhibitory horse serum.

Inhibitory sera prevented plaque formation

on chick-embryo monolayers when incorporated into the agar overlay. The inhibitory activity was stable after heating at 56 C for 30 min. The titer of WEE incubated in the presence of

FIG. 1b. *L* cells, treated as in Fig. 1a, with a 1:64 dilution of interferon. Magnification: $\times 80$.

5% IHS for 1 hr at 37 C was reduced to about 5% of that of WEE in noninhibitory serum. The above facts suggested a usefulness for IHS in interferon assays.

A test was performed to see if IHS-containing medium would selectively prevent interference by virus, yet allow interference by interferon. If it could, this would greatly facilitate interferon

FIG. 1c. *L* cells, treated as in Fig. 1a, with a 1:128 dilution of interferon. Magnification: $\times 80$.

assays, as virus would not have to be removed from interferon-containing preparations prior to their assay. The results of this test are shown in Table 5.

A virus suspension of WEE (L-) with a titer of 10^{10} PFU per ml was prepared in chick-embryo cells. It was diluted (1:10 and 1:100) in Eagle's medium containing 5% noninhibitory

TABLE 4. CPE and virus production in interferon-treated cultures of L cells^a

Expt no.	Dilution of interferon	CPE (at 72 hr)	Virus yield (PFU at 25 hr)	Virus yield %
1	1:8	—	2.3×10^5	0.3
	1:16	—	1.5×10^6	2
	1:32	+	3.0×10^6	4
	1:64	+++	1.6×10^7	22
	1:128	+++	4.3×10^7	58
	No IF	+++++	7.4×10^7	100
2	1:8	—	6.2×10^4	0.75
	1:16	—	1.3×10^5	1.6
	1:32	+	3.7×10^5	4.6
	1:64	++	2.2×10^6	27.0
	1:128	+++	3.5×10^6	44.0
	No IF	+++++	8.0×10^6	100
3	1:8	—	6.0×10^3	0.03
	1:16	—	2.1×10^4	0.11
	1:32	—	2.4×10^4	0.13
	1:64	±	2.5×10^5	1.4
	1:128	++	1.6×10^6	9.0
	1:256	+++++	1.8×10^7	100
	No IF	+++++	1.8×10^7	100

^a L-cell monolayers were treated overnight with 2.0 ml of interferon diluted as indicated in Eagle's medium containing 5% IHS. They were washed two times and challenged by the addition of more than 2.0×10^7 PFU of WEE (L+). Degeneration was scored as follows: — = none; + = 0 to 25%; ++ = 25 to 75%; +++ = 75 to 95%; ++++ = complete; ± = monolayer with floating debris in the medium but no disruption of the monolayer.

serum, and similarly diluted in medium containing 5% IHS; 2 ml of each dilution of virus suspension from the medium containing non-inhibitory serum were added, along with 0.5 ml of the same medium, to duplicate L-cell monolayers. To four monolayers, 2 ml of each dilution of virus suspension from the medium containing IHS were added; 0.5 ml of IHS-containing medium was added to two of the four monolayers at each dilution, and 0.5 ml of interferon was added to the other two. To two control monolayers, 2.5 ml of IHS-containing medium were added. They were incubated overnight at 37 C in an atmosphere of CO₂. Fluids were removed from all cells, and, after two washes, the monolayers were challenged with WEE (L+) as previously described.

TABLE 5. Assay of interferon in the presence of active virus^a

Additions prior to challenge		Immunity ^b
Virus (PFU)	Other	
WEE (L—)		
1.5 × 10 ⁹	HS	Almost complete
1.5 × 10 ⁹	IHS	None
1.5 × 10 ⁹	IHS + IF	Complete
1.5 × 10 ⁸	HS	Almost complete
1.5 × 10 ⁸	IHS	None
1.5 × 10 ⁸	IHS + IF	Complete
None	IHS	None

^a L-cell monolayers were treated overnight with 2.0 ml of the above mixtures. They were washed two times and challenged by the addition of WEE (L+). The amount of degeneration was determined 72 hr after challenge. Abbreviations used: HS, horse serum free from inhibitor; IHS, horse serum inhibitory to viral CPE; IF, interferon at a final dilution of 1:5.

^b Degree of immunity of L-cell monolayers to CPE after challenge.

It will be remembered from Table 1 that 10^8 or more PFU of WEE (L—) is capable of conferring immunity to CPE on monolayer cultures of L cells.

In the experiment described, a slight amount of degeneration occurred, estimated to be no more than 5%. This can be seen by referring to lines 1 and 3 of Table 5. Lines 2 and 5 indicate that the protection conferred by WEE (L—) was completely abolished by the addition of IHS. The presence of IHS did not, however, prevent interferon from conferring its protection independently of the virus concentration present (lines 3 and 6, Table 5).

The same selective ability to permit interferon action in the presence of virus was shown to be the case for a suspension of WEE (L+), normally cytopathogenic for L cells. A virus suspension of WEE (L+) containing 3×10^7 PFU per ml was prepared on L cells. One portion of this suspension was centrifuged several times in a Spinco model L centrifuge, and the virus titer was reduced to 3×10^4 PFU per ml. Both the centrifuged and uncentrifuged preparations were diluted in twofold steps with medium containing 5% IHS, and assayed on L-cell monolayers for their ability to produce immunity to CPE and to inhibit virus production. Both the centrifuged and noncentrifuged preparations

TABLE 6. *Production of WEE (L+) and WEE (L-) and interferon by L cells^a*

Time interval ^b	WEE (L-)		WEE (L+)	
	Virus titer	Interferon titer ^c	Virus titer	Interferon titer ^c
hr	PFU/ml		PFU/ml	
3-7	2.2×10^7	<1:2	1.3×10^8	<1:2
7-11	Lost	<1:2	Lost	1:2
11-15	1.9×10^8	1:2	1.2×10^9	1:10
15-20	1.9×10^8	1:10	2.6×10^9	1:50
20-24	1.7×10^8	1:10	1.8×10^9	1:10
24-29	3.4×10^8	1:10	1.2×10^9	1:10
29-42	3.2×10^8	1:10	4.4×10^8	1:10

^a Blake bottles of L cells containing about 30 million cells were infected with WEE (L-) and WEE (L+). After 3 hr for adsorption, the inocula were removed, and 20 ml of Eagle's medium CS-5 added. This was removed and replaced at the hourly intervals indicated above, and the fluids were assayed for infective virus and interferon.

^b Interval denotes the amount of time after addition of virus that the fluid was left in contact with the cells.

^c The interferon titer is the dilution of interferon that gave complete protection to monolayers of L cells when challenged with WEE (L+) after overnight incubation.

gave identical end points. No CPE was evident in those monolayers which received the 1:16 dilution prior to challenge. Likewise, the 25-hr yields of virus were similar throughout. Only about 0.5 to 1.0% the amount of virus produced by the controls was produced by those monolayers at the CPE end point. The presence of IHS prevented the infection of cells even when virus was present in a concentration which usually produces complete CPE. Under the conditions employed, both the protection from CPE and the inhibition of virus production were independent of the amount of virus present in the interferon-containing suspension.

To rule out further the possibility that virus in the presence of IHS was responsible for interference, a WEE (L+) stock with a titer of 2×10^9 PFU per ml was prepared in chick-embryo cells. As previously mentioned, no interferon for L cells could be demonstrated in virus stocks prepared in chick cells. The above virus suspension was diluted from 1:20 to 1:20,000 in tenfold steps in IHS-containing medium, and each dilu-

tion was assayed on L-cell monolayers for its ability to confer immunity to CPE. No protection was seen at any dilution of virus.

Production of interferon during active infection. Interferon and virus production by L cells were determined during the growth of both WEE (L+) with accompanying CPE and WEE (L-) which did not produce CPE. The experiments were carried out in Blake bottles containing about 30 million cells. Cell sheets were washed with PBS + BSA, and separate cultures were inoculated with 2×10^{10} PFU of WEE (L-) and 7×10^8 PFU of WEE (L+), respectively, in 6.0 ml of PBS + BSA. After 3 hr for adsorption, the inocula were removed, and 20 ml of Eagle's medium CS-5 added. The 20 ml of fluid were removed and replaced at about 4-hr intervals. One-half of the fluid was frozen and reserved for virus assay, and the remainder kept in a refrigerator for interferon assay. The results are presented in Table 6. Interferon was produced and released simultaneously with active virus throughout the growth cycle. In the culture infected with WEE (L+), the amount of interferon in the fluid was greatest during that 5-hr period (from 15 to 20 hr) when virus production was greatest. Interferon production did not increase as virus production became less. This is indicated by the finding that there was no significant increase in the titer of interferon in the fluids between 29 and 42 hr. Two other experiments confirmed that the interferon content was as great at 24 as at 48 hr. It appears that interferon may be produced as a by-product of virus synthesis. Although heat- and ultraviolet light-inactivated virus can act as interfering agents for L cells, I have been unable to demonstrate the presence of any interferon in the fluids of L cells treated with these agents. This may mean that interferon was not released into the medium or was in too small a quantity to be detected by the assay described. It might, however, indicate that noninfectious virus can protect cells without the mediation of interferon. Further investigations of this idea are in progress.

Production of interferon by cells treated with interferon. The preceding data suggested that interferon production by L cells was dependent on at least some steps of infection by active virus, and perhaps even related to the amount of virus synthesized. This was of special interest in light of the numerous reports (summarized by

TABLE 7. *Production of Western equine encephalomyelitis (L+) virus and interferon by interferon-treated L cells*

Expt no.	Dilution of interferon	CPE (at 72 hr)	Virus yield ^a	Interferon titer ^b
			PFU/cell	
1	1:10	—	2.3	<1:2
	1:20	—	3.8	<1:2
	1:40	—	12.0	1:10
	1:80	±	20.0	1:10
	1:160	+	100.0	1:50
	None	++++	750.0	1:10
2	1:16	—	0.002	<1:2
	1:32	—	0.003	<1:2
	1:64	—	0.02	1:2
	1:128	+	6.0	1:8
	1:256	+++	10.0	1:16
	None	++++	1,170.0	<1:2

^a Virus titers were determined for fluids harvested 24 hr after challenge in experiment 1, and after 48 hr in experiment 2.

^b Interferon titers were determined on the crude fluids harvested 24 hr after challenge in experiment 1 and after 48 hr in experiment 2. The interferon titer is the dilution of interferon that gave complete protection to monolayers of L cells when challenged with WEE (L+) after overnight incubation.

Ho, 1962) in which interferon had been produced by subjecting cells to suspensions of inactivated virus. If virus synthesis were necessary for interferon production, the amount of interferon produced by cultures of cells treated with interferon and later challenged should be related to the amount of virus produced. Table 4 indicates that the amount of virus produced by a culture of cells can be somewhat regulated by the dose of interferon added. The following experiments were done. Monolayer cultures of L cells were treated overnight with varying doses of interferon. They were washed twice and challenged by the addition of about 10 PFU of WEE (L+) per cell. Fluids were removed at 24 hr in experiment 1 and at 48 hr in experiment 2, after the addition of challenge virus. Virus and interferon contents were determined. The results are shown in Table 7. Interferon production was detectable when as little as 0.02 PFU per cell of virus was produced but interferon could not be found in cultures containing less virus. It must be remembered that WEE is

fairly heat-labile, and the 0.02 value was determined for fluids removed after 48 hr and will, therefore, most likely be smaller than the true value. An increase of interferon content was noted in those fluids containing more virus. These data, plus those presented previously, which show an apparent connection between the temporal relationships of virus and interferon production and the lack of interferon production by inactivated virus suspensions, indicate that, in the system described here, interferon may well be a by-product of virus synthesis.

DISCUSSION

It has been shown that L cells infected with a cytopathogenic and a noncytopathogenic variant of WEE produce an interferon. Interferon production appeared to be correlated, quantitatively and temporally, with virus production. In this respect, the interferon reported here is similar to the T inhibitor contained in vesicular stomatitis virus stocks (Bellett and Cooper, 1959) but different from the interferon produced by influenza virus. Interferon appears in the allantoic fluid of chick embryos infected with influenza virus much later than does active virus (Wagner, 1960). Interferon production by L cells and WEE appears to differ in another respect from interferon production by chorioallantoic membrane, or chick embryos, and influenza virus (Burke and Isaacs, 1958) in that no interferon could be found when cells were protected with either heat- or ultraviolet-inactivated viruses. This suggests that active infection is necessary for interferon synthesis in L cells. A report to the contrary is that of Paucker, Skurska, and Henle (1962), who found that L cells exposed to ultraviolet-inactivated Newcastle disease virus produce an interferon. These findings certainly do not permit one to generalize on the requirements for interferon production at the present time, but suggest that a unique relationship might exist for different viruses.

The WEE-induced interferon differs also in its heat stability from influenza interferon. Interfering activity of WEE interferon was greatly reduced after heating at 55 C for 30 min, whereas influenza interferon was completely stable at 70 C for 1 hr. It is not certain to what this difference may be attributed, but it may be only a difference in the fluids in which

the two interferons were suspended during heating. It is interesting that the WEE interferon resembles the T inhibitor produced by vesicular stomatitis virus (Bellett and Cooper, 1959) and the interferon produced by parainfluenza 3 virus (Chany, 1961) in its heat lability.

Virus stocks produced on L and chick-embryo cells did not contain cross-reacting interferon. These results agree with the data reported by Wagner (1961), Isaacs and Westwood (1959), and Tyrrell (1959) on the species-specificity of interferon. The careful choice of stock viruses enables one to separate interference by virus particles from that due to interferon.

These data extend the observation on interference in L cells produced by infection with WEE and lay the groundwork for a quantitative study of interferon action in a homologous continuous-cell system. Active virus, inactive virus, and an interferon are capable of making L cells immune to homologous WEE which normally causes complete degeneration. Similar observations in which both active virus and an inhibitor were involved were made with Mayaro virus and chick-embryo cells (Henderson and Taylor, 1961). The relative roles that interferon and inactive virus, if indeed they are separate, play in the production and maintenance of the virus carrier state are not completely clear, since each alone is capable of interfering.

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