An Antiviral Substance in the Tissues of Mice Acutely Infected with Lymphocytic Choriomeningitis Virus

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SUMMARY

Injection of mice with mouse brain suspensions of lymphocytic choriomeningitis virus produced a non-specific virus inhibitor which required 48 to 72 hr to act in monkey kidney and HeLa cell cultures. Like interferon it was active against numerous RNA viruses, was non-sedimentable, was unaffected by antiserum to lymphocytic choriomeningitis virus, and showed a dose-response effect. However, unlike interferon it was acid-labile, crossed species barriers, and was eliminated by changing the medium at the time of virus challenge. Mouse brain preparations freed of detectable virus by ultracentrifugation had properties similar to preparations containing lymphocytic choriomeningitis virus. Furthermore, tissue culture fluids containing lymphocytic choriomeningitis virus contained no inhibitor. Tissues of mice infected with the WE strain of lymphocytic choriomeningitis virus also contained no inhibitor. Hence, this inhibitor appears to represent a new class of broad spectrum virus inhibitor that may be useful for the characterization of lymphocytic choriomeningitis virus strains.

INTRODUCTION

The first report of heterologous virus interference produced by lymphocytic choriomeningitis (LCM) virus indicated that monkeys inoculated subcutaneously with LCM virus were resistant to a normally lethal strain of poliovirus (Dalldorf, 1939). Based on this report, an unsuccessful attempt was made to protect HeLa cells against the destructive effects of poliovirus by infecting first with LCM virus (Hotchin & Cinits, 1958). Another attempt to show such interference in murine lymph node cultures infected with LCM virus also failed (Traub & Kesting, 1963). More recently, heterologous interference of an intrinsic nature (Marcus & Carver, 1965, 1967) was demonstrated in mouse embryo and chick embryo cell cultures infected with the ARMSTRONG or WE-3 strains of LCM virus (Wainwright & Mims, 1967). Although heterologous interference has been produced by LCM virus in vivo (Dalldorf, 1939; Jungeblut & Kodza, 1963; Traub, 1961; Wainwright & Mims, 1967; Lewis & Clayton, 1969), there is no reported evidence that interferon detectable by cell culture methods is induced by LCM virus. This communication describes an inhibitor in tissue homogenates from mice acutely infected with LCM virus.

METHODS

Tissue culture. African green monkey kidney (GMK), serial L-929 mouse fibroblasts, HeLa, BHK 21 (Clone 13) and primary chick embryo cell cultures were grown in glass roller tubes (16 × 125 mm.). The L-929 mouse fibroblasts, GMK, and HeLa cells were
grown in Eagle's minimal essential medium with 10% calf serum. The BHK 21 and chick embryo cells were grown in Eagle's basal medium in Hanks's salts with 10% tryptose phosphate broth and 10% foetal calf serum. Virus inhibitor assays were carried out in similar media with the following changes in serum concentrations: 0.5% calf serum in GMK medium, 4% calf serum in the L-929 mouse fibroblast and HeLa media, and 2% foetal calf serum in the BHK 21 and chick embryo media.

All cell lines were monitored weekly for *Mycoplasma* on Chanock's medium (Chanock, Hayflick & Barile, 1962). These plates were incubated anaerobically at 37°C for 2 to 3 weeks. Primary cell cultures were tested as above using culture fluids from individual experiments.

**Viruses.** The ARMSTRONG strain of LCM virus (VR-134), received from the American Type Culture Collection, was used in all experiments, except where otherwise indicated. The virus has been passed six times in monkeys and about 209 times in mice. Stock virus was prepared as a 20% mouse brain suspension (w/v) in a diluent consisting of equal volumes of tryptose phosphate broth and skim milk. This suspension was stored at -70°C. The 20% suspension was always diluted to 10% and clarified by low speed centrifugation before use in an experiment. *Mycoplasma* were assayed for as above.

The WE strain of LCM virus was provided by Dr J. C. Winn of the National Communicable Disease Centre. This virus has been passed eight times in guinea-pig brain and five times in mouse brain. Virus stocks were prepared and assayed for *Mycoplasma* as above.

The SABIN strain of poliovirus type 1 was derived from a sample of oral vaccine. Stock virus preparations consisted of GMK cell culture fluids. Following clarification by centrifugation, these fluids were stored at -70°C until needed.

The INDIANA strain of vesicular stomatitis virus (VSV) was provided by Dr J. V. Hallum of the University of Pittsburgh School of Medicine. This virus and all others were prepared as poliovirus type 1 above.

**Virus assays.** LCM virus was titrated in randomly bred white Swiss mice. Serial tenfold dilutions were prepared and 0.03 ml. was injected intracerebrally into groups of five mice. The fatal LCM disease syndrome was the indicator of LCM virus infection. Mice were observed for a minimum of 10 days after infection.

All other viruses were titrated in the cell cultures in which they were prepared using five roller tubes/serial tenfold dilution. Haemadsorption served as the index of infection for myxoviruses; cytopathic effect was used for all others.

**Inhibitor assay.** The assay is based on the fact that LCM virus replicates but does not produce observable cytopathic effects in cell cultures. However, the challenge viruses caused complete cytopathic effect or haemadsorption in 48 to 96 hr. Except where indicated, inhibitor assays were performed as follows in all cell culture systems. On day zero, growth medium from freshly sheeted roller tubes was replaced with inhibitor assay medium and 1000 LD50 of LCM mouse brain suspension was added per tube. Following incubation at 37°C these cultures and uninfected controls were challenged daily with 1000 TCD50 of the challenge virus without performing a medium change. After further incubation at 37°C, and when challenge virus controls showed complete cytopathic effect or haemadsorption, the degree of inhibition was assessed in LCM mouse brain infected cell cultures. A 50% reduction in the degree of cytopathic effect or haemadsorption was considered significant (Finter, 1966; Wagner, Levy & Smith, 1968). At this time, usually 48 hr after challenge, culture fluids from test and control tubes were pooled and stored at -70°C. All references to time following addition of inhibitor refer to the day on which cultures were challenged.

**Inhibitor preparation.** Using the method of Finter (1964), a 10% LCM mouse brain suspension in Hanks's balanced salt solution (HBSS) was clarified at 4340 g for 30 min. in
Inhibitor of LCM virus-infected mice

the cold. The supernatant fluid was collected and centrifuged at 110,000 g for 3 hr in the cold. This supernatant fluid was collected and recentrifuged at 110,000 g as above. All but the bottom fourth of this supernatant fluid was collected. The supernatant fluids were designated ARM-S-2 for ARMSTRONG strain or WE-S-2 for WE strain.

RESULTS

Preliminary experiments showed that the titration of poliovirus infectivity was unaffected by the presence of lymphocytic choriomeningitis virus in those same cell culture fluids. Serial tenfold dilutions of poliovirus in HBSS with or without 1000 LD50/0.2 ml. of LCM mouse brain suspension yielded similar poliovirus titres. Furthermore, the reverse was also shown to hold—poliovirus had no effect on infectivity of LCM virus for mice. Serial tenfold dilutions of LCM virus were made in HBSS containing approximately 1000 TCD50/0.03 ml. of poliovirus and 20 % antipoliovirus rabbit serum or normal rabbit serum and incubated for 1 hr at room temperature before inoculation into mice. Table 1 indicates that the presence of poliovirus did not affect the titres of LCM virus. Addition of antiserum to poliovirus was unnecessary for such titrations.

Table 1. Mouse infectivity of lymphocytic choriomeningitis virus in fluids containing poliovirus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Serum</th>
<th>Log. LD50/0.2 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCM</td>
<td>Normal rabbit</td>
<td>5.33</td>
</tr>
<tr>
<td>LCM</td>
<td>Antipolio</td>
<td>5.33</td>
</tr>
<tr>
<td>LCM + Polio-1</td>
<td>Normal rabbit</td>
<td>5.15</td>
</tr>
<tr>
<td>LCM + Polio-1</td>
<td>Antipolio</td>
<td>5.33</td>
</tr>
</tbody>
</table>

Armstrong mouse brain effect on challenge viruses

Inhibition of poliovirus synthesis by standard dose of LCM virus

Green monkey kidney cell cultures inoculated with 1000 LD50 of LCM mouse brain suspension/tube on day zero were challenged daily for 12 days with 1000 TCD50 of poliovirus. Interference in the mixed virus system was noted 48 hr after addition of challenge virus. At this time the cell culture fluids from poliovirus controls, LCM virus controls, and the mixed system were pooled.

Fig. 1 shows inhibition started on day 3, indicated by a decrease in poliovirus yield and inhibition of cytopathic effect. These effects reached a maximum on the 5th day of challenge when poliovirus yield was reduced by 99.99 %. The inhibitory effect began to decline on day 6 and continued to do so until day 8. Inhibition of poliovirus yield correlated well with the inhibition of poliovirus cytopathic effect indicated at the bottom of Fig. 1.

In addition to the reduction in poliovirus yield in doubly infected cells, a reduction in LCM virus production in the same cells became evident on day 6 and reached a maximum on day 8 when the yield of LCM virus was decreased by 95.5 %. It should be noted that poliovirus cytopathic effects increased continuously from day 6 to day 8. Inhibition of poliovirus was no longer evident in doubly infected cells by day 8, although inhibition of LCM virus in the same cells persisted throughout the 12 day test period.

Inhibition of VSV synthesis by standard dose of LCM virus

GMK cultures inoculated with LCM mouse brain suspensions as above and challenged daily with vesicular stomatitis virus showed an inhibition of virus yield and cytopathic
effects (Fig. 2). Maximum inhibition of VSV yield (99.5%) and cytopathic effect occurred on day 5. Beginning on day 6, inhibition of VSV yield began to decline concomitant with increasing VSV cytopathic effects through day 8. On day 9 another drop in VSV yield and cytopathic effect occurred. The time for onset of inhibition, the day of maximum effectiveness, and also the decrease beginning on day 6 correlated well with the poliovirus system shown in Fig. 1.

![Graph showing inhibition of poliovirus yield and cytopathic effect by lymphocytic choriomeningitis mouse brain suspension in GMK cell cultures.](image)

Fig. 1. Inhibition of poliovirus yield and cytopathic effect by lymphocytic choriomeningitis mouse brain suspension in GMK cell cultures. Culture medium was replaced on day 8. Days indicate time of addition of poliovirus to test and control cultures. ●●●, poliovirus only; ○○○, poliovirus in presence of LCM virus; ▲▲▲, LCM virus only; △△△, LCM virus in presence of poliovirus. Open bars indicate the degree of cytopathic effect developing in cultures containing LCM virus and poliovirus. Closed bars indicate the degree of cytopathic effect in cultures with poliovirus only.

**LCM virus dose needed to inhibit poliovirus**

To evaluate a dose-response effect relationship, dilutions of LCM mouse brain suspension calculated to contain 10, 100 or 1000 LD50 of LCM virus were inoculated into GMK cell cultures which were challenged daily with 1000 TCD50 of poliovirus. At doses of 100 and 1000 LD50 of LCM virus, the yield of poliovirus was reduced significantly beginning on day 4 and day 3, respectively (Fig. 3). The 1000 LD50 dose of LCM virus was more efficient in reducing poliovirus yield, at least until day 8. No inhibition of poliovirus yield or cytopathic effects occurred with the 10 LD50 LCM virus dose. Thus, the minimum inhibiting dose was between 10 and 100 infectious doses of LCM virus.
Inhibitor of LCM virus-infected mice

The duration of inhibition of poliovirus cytopathic effects was also followed in this experiment. Culture medium was replaced with fresh medium after the inhibition of cytopathic effect was noted. In cultures challenged on day 5, when maximum interference was demonstrated, the inhibition of poliovirus cytopathic effect persisted 5 to 7 days. On days preceding the 5th day of challenge, the duration of inhibition of poliovirus cytopathic effect was shorter, being usually 3 to 4 days. Also, inhibition of cytopathic effects was longest in GMK tubes inoculated with the 1000 LD50 dose of LCM virus.

Viruses inhibited by LCM mouse brain suspensions

GMK roller tubes were inoculated with LCM mouse brain suspension and incubated for 5 days before addition of 1000 TCD50 of challenge viruses listed in Table 2. When virus controls showed complete cytopathic effect or haemadsorption, the degree of inhibition in the mixed virus systems was estimated. Of the viruses tested, only vaccinia and reovirus type 1 were not inhibited.
**Distribution of inhibitor in tissues of acutely infected mice**

In order to demonstrate the inhibitor in tissues of the mouse other than the brain, 50 mice were inoculated with the ARMSTRONG strain of LCM virus. After 5 days the brain, liver, kidney and spleen were homogenized, suspended to 10% (w/v) and centrifuged at 4340 g. Dilutions of tissues and serum were inoculated into GMK cultures. Five days later the cells were challenged with poliovirus and the cytopathic effect was scored 48 hr later.

**Table 2. Inhibition of heterologous viruses in monkey kidney cell cultures by lymphocytic choriomeningitis mouse brain suspensions**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Degree</th>
<th>Time for onset (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coxsackie A-9</td>
<td>++</td>
<td>48</td>
</tr>
<tr>
<td>Coxsackie B-1</td>
<td>++</td>
<td>48</td>
</tr>
<tr>
<td>Coxsackie B-2</td>
<td>++</td>
<td>48</td>
</tr>
<tr>
<td>Coxsackie B-3</td>
<td>+</td>
<td>72</td>
</tr>
<tr>
<td>Coxsackie B-5</td>
<td>++</td>
<td>120</td>
</tr>
<tr>
<td>Coxsackie B-6</td>
<td>+</td>
<td>72</td>
</tr>
<tr>
<td>Echo-11</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>Poliovirus-1</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>VSV (INDIANA)</td>
<td>+</td>
<td>24</td>
</tr>
<tr>
<td>Influenza (PR-8)</td>
<td>+</td>
<td>72</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>o</td>
<td>0</td>
</tr>
<tr>
<td>Reovirus-1</td>
<td>o</td>
<td>0</td>
</tr>
</tbody>
</table>

* + + = 100% Inhibition of CPE; + = 75% inhibition of CPE; o = no inhibition of CPE.

**Table 3. Inhibitor in tissues of mice acutely infected with the ARMSTRONG strain of lymphocytic choriomeningitis virus**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Tissue*</th>
<th>Inhibitor doses†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Brain</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.00†</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>2.05</td>
</tr>
<tr>
<td>2</td>
<td>Brain</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>1.55</td>
</tr>
<tr>
<td>3</td>
<td>Brain</td>
<td>2.55</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>2.05</td>
</tr>
</tbody>
</table>

* All tissues were 10% (w/v) homogenates which were further diluted in HBSS before inoculation into cell cultures.
† Log 50% inhibitory doses/0.2 ml. of 10% tissue suspension or undiluted serum based on inhibition of cytopathic effect of poliovirus.
‡ No activity at a 1:10 dilution.

Log 50% inhibitory titres were calculated by the method of Reed & Muench (1938). Serum and tissues, except liver, contained inhibitor (Table 3). Brain tissue has consistently shown greater inhibitory activity/unit weight than other tissues. Brain and other tissues were ten times more active than serum, since a 10% tissue suspension served as the starting material for the former.
Inhibitor of LCM virus-infected mice

Inhibition of poliovirus in HeLa cells

HeLa cell cultures were inoculated with 2000 LD50 of LCM mouse brain suspension/tube and subsequently challenged daily with 1000 TCD50 of poliovirus. A one-half medium change using fresh LCM virus-free medium was performed on days 2, 4 and 5. Maximum inhibition of poliovirus yield (98%) and cytopathic effect occurred in cultures challenged on day 4 (Fig. 4). The inhibition continued throughout the duration of the experiment but to a lesser extent than observed on day 4.

![Graph](image)

Fig. 4. Inhibition of poliovirus yield by lymphocytic choriomeningitis mouse brain suspension. Groups of HeLa cell cultures, either treated (○—○) or untreated (●—●), were challenged daily with poliovirus. Days indicate the time of addition of poliovirus. Half the culture medium was replaced on days 2, 4 and 5.

Further characterization of effect

Inability of \textit{we} strain of LCM virus to inhibit poliovirus

GMK cell cultures were inoculated with 1000 LD50 of LCM mouse brain suspension, \textit{we} strain, and challenged daily with 1000 TCD50 poliovirus. When poliovirus controls showed complete cytopathic effect, the inhibitory effect was assessed in LCM virus-infected tubes. Inhibition of neither poliovirus yield nor cytopathic effect was demonstrated. Similarly, neither serum nor suspensions of liver, kidney, or spleen prepared as above from mice infected with the \textit{we} strain inhibited the cytopathic effect of poliovirus.

Role of normal mouse brain constituents

A 10% normal mouse brain suspension (w/v) prepared in skimmed milk and tryptose phosphate broth (1:1) was inoculated into GMK cell cultures. After incubation for 5 days, both treated and control cultures were challenged with 1000 TCD50 poliovirus. Normal mouse brain failed to inhibit poliovirus yield or cytopathic effect and hence was not responsible for the inhibition observed with LCM mouse brain suspensions.

Inability of GMK-grown LCM virus to inhibit poliovirus

Undiluted GMK cell culture fluid containing either the \textit{ARMSTRONG} or \textit{we} strain of LCM virus was inoculated into GMK roller tubes at about 10,000 LD50/tube. Five days later poliovirus was added to the tubes inoculated with LCM virus and to control tubes. No inhibition of poliovirus yield or cytopathic effect occurred.
Effect of a complete medium change on inhibition

GMK roller tubes inoculated with tenfold dilutions of LCM mouse brain suspension were divided into two groups and incubated for 5 days. At the end of this time, challenge virus was added directly to one group, whereas the medium in the second group was drained and replaced with fresh medium. Challenge virus was added to both systems and controls as above. End points were determined as above based on cytopathic changes on haemadsorption. The medium change which removed the LCM mouse brain suspension also eliminated the inhibitory effect (Table 4). Poliovirus proved to be the most sensitive assay virus for detection of LCM inhibitor.

Table 4. Effect of a medium change before the addition of challenge virus on inhibitory activity

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Medium change</th>
<th>Inhibitory doses†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus-1</td>
<td>+ *</td>
<td>0</td>
</tr>
<tr>
<td>Poliovirus-1</td>
<td>-</td>
<td>5.80</td>
</tr>
<tr>
<td>Echo-11</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Echo-11</td>
<td>-</td>
<td>4.47</td>
</tr>
<tr>
<td>Parainfluenza-1</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Parainfluenza-1</td>
<td>-</td>
<td>5.13</td>
</tr>
</tbody>
</table>

* +, Indicates a complete replacement of maintenance medium at time of challenge; -, indicates challenge viruses were added directly to medium already on cultures.
† Log. 50 % inhibitory doses/0.2 ml. of LCM mouse brain suspension based on inhibition of cytopathic effect or haemadsorption.
‡ No activity at a 1:10 dilution of LCM mouse brain suspension.

Table 5. Cell and species specificity of the inhibitor in lymphocytic choriomeningitis virus-infected mouse brain

<table>
<thead>
<tr>
<th>Cell cultures</th>
<th>LCM(ARM)</th>
<th>LCM(WE)</th>
<th>LCM(ARM-S-2)</th>
<th>LCM(WE-S-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMK</td>
<td>++ †</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>HeLa</td>
<td>+ †</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Mouse embryo</td>
<td>+ + †</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-929</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BHK 21 (clone 13)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* LCM(ARM) and LCM(WE) are 10 % mouse brain suspensions of ARMSTRONG and WE strains of LCM virus and were diluted 1:100 in HBSS before inoculation into cell cultures. LCM(ARM-S-2) and LCM(WE-S-2) are virus-free preparations of LCM(ARM) and LCM(WE) made by ultracentrifugation and were diluted 1:10 in HBSS before inoculation into cell cultures.
† 100 % inhibition of CPE in each of five roller tubes challenged with VSV on day 5.
‡ 50 % inhibition of CPE in each of five roller tubes.

Cell and species specificity of inhibitor

A qualitative comparison of the susceptibility of various cell cultures to the action of LCM mouse brain inhibitor was made. Undiluted mouse brain inhibitor preparations from the second cycle of ultracentrifugation (ARM-S-2 and WE-S-2, described above) when injected intracerebrally into mice did not cause LCM disease and were considered to be free of infectious virus. These preparations were diluted 1:10 in HBSS, whereas the WE and ARMSTRONG strains of LCM mouse brain suspensions (10 % w/v) were diluted 1:100 before inoculation into cell cultures. No medium change was performed on primary cell cultures,
but a one-half fresh medium change was performed on cell lines on day 3. All cell culture systems were challenged on day 5 with VSV. Inhibitor activity was demonstrated with both ARMSTRONG strain preparations in three of six cell types surveyed (Table 5). However, neither the WE-S-2 nor WE mouse brain suspension of LCM virus showed activity in any cell culture system.

A quantitative comparison of the inhibitor activity in the sensitive cell cultures was performed. Serial tenfold dilutions of LCM mouse brain suspension were inoculated into roller tube cultures and challenged 5 days later with a standard dose of VSV. Cytopathic end points were determined and the inhibitory titre thereby calculated for each cell type. The inhibitor was most active in mouse embryo cell cultures (6.86 InD50/0.2 ml.), less in GMK cell cultures (4.52 InD50/0.2 ml.), and least activity occurred in HeLa cell cultures (3.32 InD50/0.2 ml.).

Effect of LCM antiserum on inhibition

The effect of antibody to LCM virus was determined by an interference assay in GMK cell cultures. The antiserum was prepared according to the method of Bucca (1964) and had a complement fixation titre of 1:32. Antiserum was diluted 1:10 either in HBSS or in HBSS supplemented with 20% fresh guinea-pig serum. Equal parts of antiserum or diluent were mixed with LCM mouse brain suspension containing 160 LD50 of virus/0.5 ml. After incubation at room temperature for 1 hr, 0.5 ml. of each mixture was added to GMK cultures. Serum, virus and inhibitor controls were also maintained. All systems were challenged 5 days later with a standard dose of poliovirus. Neither antibody to LCM virus nor antibody with fresh guinea-pig serum prevented the inhibitory effect produced by LCM mouse brain suspension.

Acid lability of the LCM inhibitor

An attempt was made to purify the inhibitor using methods applied to classical vertebrate interferons (Lampson et al. 1963). However, both virus and inhibitor were inactivated at pH 2.0 during perchloric acid precipitation. Further attempts at purification were therefore discontinued at this time.

DISCUSSION

We have shown that there is an antiviral substance in the tissues and serum of mice acutely infected with the ARMSTRONG but not the WE strain of LCM virus. Such strain differences, though uncommon among non-specific virus inhibitors (Wasserman, 1968), are commonplace among virus- and non-virus-induced interferons (Finter, 1966; Friedman, Rabson & Kirkham, 1963; Lockart, 1963). Strain differences of viruses have been attributed to virulence factors (Ruiz-Gomez & Isaacs, 1963). Together with other strain differences reported for LCM virus (Camyre & Pfau, 1968) the detection of the inhibitor in LCM mouse brain virus stocks serves as a useful criterion for characterization of additional LCM virus strains and provides in vitro substantiation for experiments of Dalldorf (1939).

The fact that the inhibitor acted similarly in suppressing synthesis and cytopathic effects of biologically different viruses such as poliovirus and VSV tends to rule out a receptor site interference. Furthermore, the ability of the inhibitor to interfere with a wide spectrum of viruses would discourage its consideration as a substance which neutralizes the infectivity of challenge viruses (Wasserman, 1968).

Alternatively, the interference observed was similar to the intrinsic interference described by Marcus & Carver (1965) in rubella virus-infected GMK cell cultures. Elimination of
intrinsic interference by a medium change was attributed to removal of an extrinsic factor. Further studies demonstrated a requirement for virus-coded protein synthesis (Marcus & Carver, 1968). Also, Wainwright & Mims (1967) described an interference test for assaying LCM virus using the haemadsorption-negative plaque assay and related it to the intrinsic interference effect of Marcus & Carver (1965). However, since intrinsic interference requires function of the virus genome, any attempt to relate it to the LCM virus inhibitor above is discouraged, because LCM virus was not necessary to produce the interference effect.

A survey of the properties of the LCM inhibitor indicates that it cannot qualify as an interferon or as a non-specific neutralizing factor. Its action on a wide spectrum of viruses, dose-response kinetics and non-sedimentability at 110,000 g are properties characteristic of known interferons (Lockart, 1966; Wagner et al. 1968; Finter, 1966). However, the ability of the inhibitor to cross-species barriers, loss of activity following a medium change and its inactivation at pH 2–0 are not considered properties of interferon. Furthermore, the presence of the inhibitor in virus-free preparations of infected mouse brain and insusceptibility to LCM virus antibody are evidence that the inhibitory effect is independent of the action of the virus genome.

All attempts to relate the LCM virus inhibitor of mice to classical vertebrate interferons have failed due to certain unique features of the inhibitor. Further purification, as well as biological and biochemical characterization, must be developed in accord with these features. Continued research on such inhibitors may contribute to our knowledge of non-interferon-mediated interference systems (Baron, 1966). Also, failures to demonstrate inhibitors in LCM virus-infected mice in the past may have been due to limitations of methodology employed.

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REFERENCES

Inhibitor of LCM virus-infected mice


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