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DEPARTMENT OF THE ARMY  
Fort Detrick  
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Authors: P. Gevaudan, J. Charrel, and G. Pieroni, Laboratory of Hygiene,  
Faculty of Medicine (Laboratoire d'Hygiene, Faculté de Médecine),  
Marseilles, France.

Title: Interference between Cocksackie viruses of the same type (Interference  
entre virus Cocksackie de même type).

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(1967).

April 1969

The phenomenon of interference between polio viruses I and II or between polio virus and Coxsackie virus has been observed under those conditions where each of these strains are capable of multiplying (1, 2). More recently, it has been demonstrated that a strain of type 1 polio virus, incapable of developing at  $39.5^{\circ}$  or in the presence of guanidine was, nevertheless, capable of inhibiting the development of another strain of Polio virus I which was characterized by its resistance at  $39.5^{\circ}$  and to the presence of guanidine (3).

In the two cases, the inhibiting action was different in that, in one case, the virus replicated while in the other, it did not. In the first case, inhibition takes place slowly at the time of replication of the virus. In the second case, it is rapid and occurs before synthesis of the viral nucleic acid.

Having conceded that we are employing "variants" of the Coxsackie virus for which we have measured the sensitivity to development at that temperature (SDT) (4), it was of interest to us to determine in a susceptible system the establishment of interference between Coxsackie "variants" of the same type.

#### TECHNIQUES

1. Virus Strains - A strain of thermosensitive coxsackie virus B5 and a "hot variant" obtained from it were utilized (5).

The values for the SDT were as follows: (6)

"Challenger" virus: Coxsackie B5 small plaques  $37^{\circ}$  (3);  $tr^{+}$   $35^{\circ}$ , to  $37^{\circ} \pm 0.5^{\circ}$ ;  $tr^{-}$   $38.7^{\circ}$ .

Interfering virus: Coxsackie B5 large plaques  $41.2^{\circ}$  (15);  $tr^{-}$   $36.5^{\circ}$ ; to  $39^{\circ} \pm 0.25^{\circ}$ ;  $tr^{-}$   $41^{\circ}$ .

These two clones were developed on KB cells. The virus lots to be employed in the experiments were stored in ampoules at  $-70^{\circ}$ . The titer was expressed as the PFU (plaque forming units) per ml and was determined by the method of Cooper.

2. Media - The medium employed in the titration method of Cooper has been previously described (7).

The culture medium is a variation of the classical medium given by Riordan and Lepine. It is composed on lactalbumin in Earle's solution enriched with B vitamins and young horse serum.

#### PRINCIPLE OF THE EXPERIMENT

We have set up a simple system in which the mechanism of reproduction of the interfering virus is reduced by 95 % at the temperature of incubation. This is fixed at 36°. It corresponds to the optimal temperature of development of the "Challenger" virus and also to a temperature which is 0.5° less than the  $t_r^*$  of the interfering virus (see the SDT of the respective strains).

In a manner paralleling the interference cycle were run also a normal independent cycle for the "challenger" virus and a normal independent cycle for the interfering virus.

For the purpose of clarity, the course of viral development of the interfering virus incubated at its optimal temperature, which was  $39^\circ \pm 0.25^\circ$ , does not appear on the graph since it is easily confused with that of the normal independent cycle of the "challenger" virus. The comparison of the development curves of the various cycles allows for a direct estimation of the degree of interference produced under a variety of experimental conditions. Titration of the samples was carried out using the method of Cooper (7) modified to a temperature of 37°.

#### COURSE OF AN EXPERIMENT

1. Assay of Interference - For the exclusion of an incubation period of 36°, all of the manipulations were carried out at a temperature of 22°. At this temperature, neither the "challenger" virus nor the interfering virus are capable of developing.

Into a centrifuge tube were placed 10 million KB cells suspended in 1 ml of Earle's solution. 1 ml of a suspension of interfering virus, whose titer had been previously determined, was added. The mixture was kept suspended by pipetting for a period of 15 minutes. Three minutes before completion of this period of time, the mixture was centrifuged at 500 rpm for 3 minutes and the supernatant was discarded. The sediment was rinsed by pipetting with 5 ml of Earle's solution. After recentrifugation, the supernatant was discarded. The cell pellet was recovered and suspended in 5 ml of maintenance medium. The cell suspension was transferred to a ground-glass ampoule with a fitted ground glass stopper which was coated with silicone grease at the neck.

The bottle was immediately attached to a mobile support and submerged in a water bath at a fixed temperature. Rotary agitation was carried out by a horizontal circular movement of 70 rpm. The duration of this primary incubation was varied from one experiment to another. At the end of the incubation period, the bottle was removed from the water bath and the contents were cooled to 22°. The cell suspension was centrifuged and rinsed with Earle's solution as previously described. The cells were suspended in 1 ml of Earle's solution and 1 ml of "challenger" virus, whose titer had been previously determined, was added. After a period of contact of 15 minutes, the cells were rinsed again with Earle's solution and suspended finally in 5 ml of maintenance medium which was placed in a siliconed bottle. This was incubated in a water bath at the temperature desired and was subjected to a rotary horizontal agitation of 70 rpm during the entire period of virus development. At hourly intervals, an aliquot of the mixture was removed, cooled to -60°, and stored at -70° until it was titered.

2. Control Assay of The Interfering Virus - To 10 million KB cells suspended in 1 ml of Earle's solution was added 1 ml of a suspension of interfering virus whose titer had been previously determined. Contact was maintained for 15 minutes after which time the cells were recovered by centrifugation, washed as previously described, and suspended in 5 ml of medium. The suspension was incubated in a siliconed bottle in a water bath at the suitable temperature of 36° with rotary agitation. The development of the virus was followed on an hour-by-hour basis during the entire cycle using the techniques previously described for the determination of interference.
3. Control Assay of The "Challenger" Virus - One obtains a unique cycle for the "challenger" virus under the conditions identical to those of the interfering virus.

#### RESULTS

1. Variation in the degree of interference as a function of the ratio of interfering virus to "challenger" virus - Three series of experiments were carried out each varying from the other in the quantity of "challenger" virus employed. The dose of interfering virus was kept constant at 1 PFU per cell. In the first series, the dose of "challenger" virus was 1 PFU per cell. In the second, it was 10 PFU per cell. In the third series, it was 100 PFU per cell.

Figure 1 demonstrates that the degree of interference varies as a function of the ratio of the quantities of virus employed in each case.

By employing lead-times with the interfering virus of 30 or 60 minutes, one observed: (1) that interference is <sup>complete</sup> ~~partial~~ when the dose of "challenger" virus and interfering virus are equal; (2) That interference is partial when the dose of the "challenger" virus is ten times higher than that of the interfering virus; (3) that no interference is produced when the "challenger" virus dose is 100 times higher than that of the interfering virus.

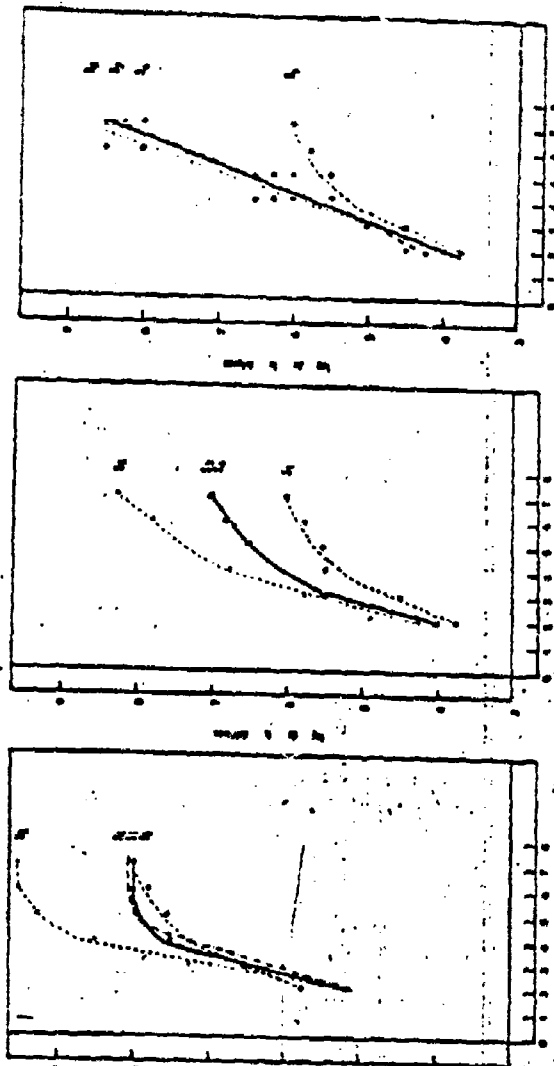


Fig. 1. Interference as a function of the ratio of the quantity of interfering virus to inhibited virus.

O — normal cycle of virus at 41°  
 O — normal cycle of virus at 37°  
 Δ — Interfering action of 41° virus on 37° virus. Temperature at start corresponds to interfering 41° virus: 30 minutes.  
 □ — Interfering action of 41° virus on 37° virus. Temperature at start corresponds to interfering 41° virus: 60 minutes

- 1 a: 41° interfering virus = 1 PFU/1 cell  
37° "Challenger" virus = 1 PFU/1 cell
- 1 b: 41° interfering virus = 1 PFU/1 cell  
37° "challenger" virus = 10 PFU/1 cell
- 1 c: 41° interfering virus = 1 PFU/1 cell  
37° "challenger" virus = 100 PFU/1 cell



(2) Variation in the degree of interference as a function of the time in advance given to the interfering virus - In the preliminary tests, we demonstrated that interference is produced by doses of interfering virus which are less than the number of cells involved in terms of PFU. As a result, we intentionally selected the weak dose of 1 PFU per 10 cells both for the interfering virus and the "challenger" virus in the experiment, where only the lead-time accorded to the interfering virus was varied.

It is demonstrated in Figure 2 that (1) simultaneous adsorption of the two viruses does not produce any interference; (2) a lead-time of 15 minutes for the interfering virus is sufficient to produce interference to that obtained when the lead time is 30 minutes; (3) when the lead-time is increased to 60 minutes, the degree of interference is very marked and is about 90 %. Thus, under the conditions of these experiments, interference appears to occur quite early in the cycle of virus development.

3. Separation of plaque-forming infectivity and the interfering capacity. -

The production of interference by a number of PFU less than the number of cells implies the presence of particles possessing interfering activity but lacking the potential to form plaques. This observation required the necessity for an enumeration assay. This was carried out during the course of the interference experiments by varying the number of cells used and keeping the dose of the interfering and "challenger" viruses fixed.

Figure 3 demonstrates that (1) for 1 PFU per 10 cells and per 20 cells, the interfering capacity is pronounced; (2) that interference is still detectable with 1 PFU per 40 cells; (3) that it is lost at 1 PFU per 60 cells.

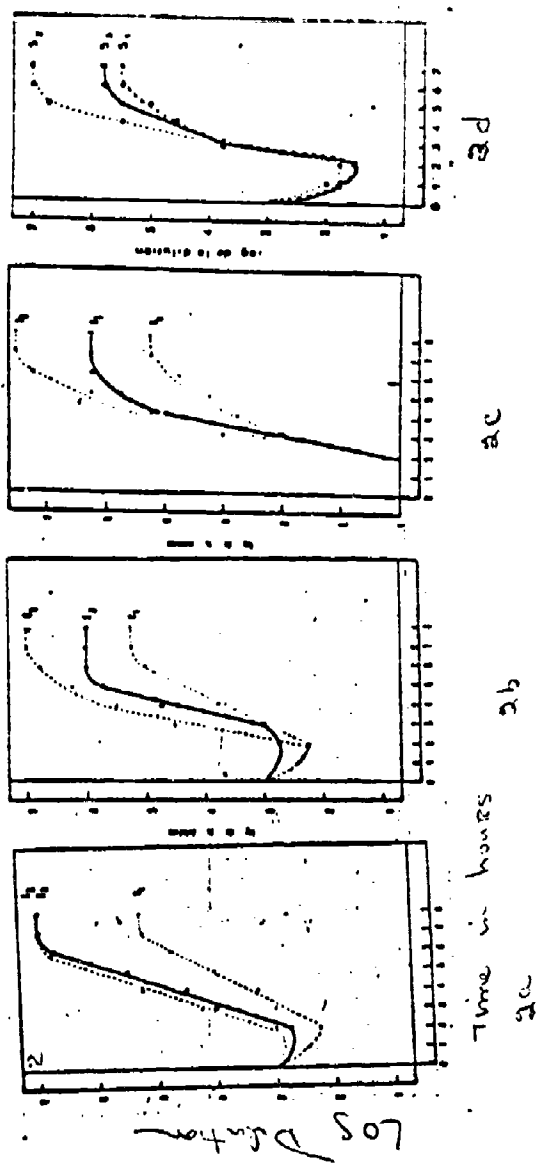


Fig. 2. Interference as a function of the lead time given to the interfering virus.

○ ..... ○ S1 = control cycle of 41° virus.  
 ○ --- ○ S2 = Control cycle of 37° virus.  
 Δ ..... Δ S3 = Interfering action of 41° virus on 37° virus.

2a : Lead-time given to interfering virus (41°) = 0 minutes

2b : Lead-time given to interfering 41° virus = 15 minutes

2c : Lead-time given to interfering 41° virus = 30 minutes

2d : Lead time given to interfering 41° virus = 60 minutes

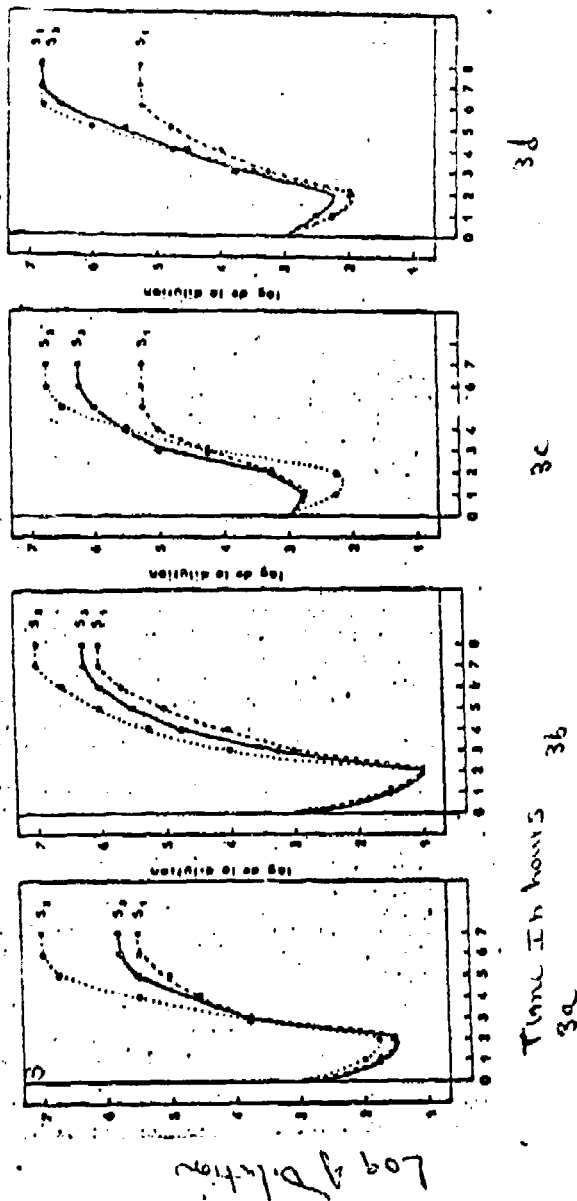


Figure 3. Interference as a function of the ratio of interfering and "challenger" viruses to the number of cells employed.

- S1 = control cycle of 41° virus.
  - S2 = control cycle of 37° virus.
  - S3 = interfering action of 41° virus on 37° virus.
- 3a: 1 PFU of interfering and "challenger" viruses per 10 cells
- 3b: 1 PFU of interfering and "challenger" virus per 20 cells
- 3c: 1 PFU of interfering and "challenger" virus per 40 cells
- 3d: 1 PFU of interfering and "challenger" virus per 60 cells

## DISCUSSION

### 1. Proof for the interference phenomenon between strains of the same type -

Based on a consideration of the SBT of the interfering virus, we chose a temperature where the reduction of its mechanism of reproduction approached 95 %.

The principle of our experiment, as a consequence, was intermediary between those carried out by Ledinko (1) and Corde and Holland (2) who allowed interfering strains to develop and those of Pohjanpelto and Cooper (3) who did not allow the interfering strain to develop.

Nevertheless, our results are comparable to those obtained by these other authors particularly with regards to the following:

(1) With weak doses of interfering and "challenger" virus, 15 minutes of lead-time is sufficient to produce about 50 % of interference.

(2) The interference produced by small amounts of interfering virus can be overcome. This is still possible even after a lead-time of 60 minutes for the interfering virus when the quantity of "challenger" virus is increased sufficiently.

It is obvious, therefore, that a strain of interfering virus, restricted to slow development, during the first 15 minutes of its cycle of reproduction is capable of restricting the development of a second virus of the same type.

### 2. Separation of Plaque-forming infectivity and Interfering capabilities -

With regards to the interference produced by non-plaque forming particles, their relative importance is high when one employs small quantities of virus. Our observations have confirmed, therefore, in a simple manner, that the viral clonal population has a composite functional condition (8,9).

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