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Letter to the Editors

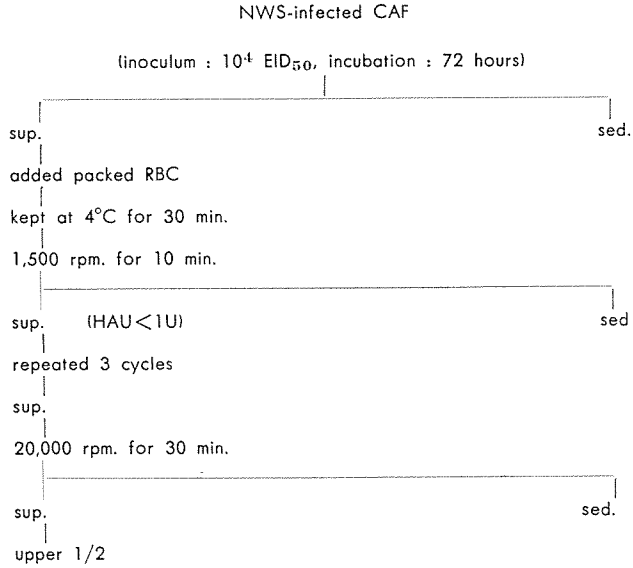
Interfering Substance Produced in the Chorioallantoic Fluid Infected with Influenza Virus Strain NWS.

It has been reported by Isaacs *et al.* that interferon is produced by the interaction between the chicken chorioallantoic membrane (CAM) and inactivated influenza virus, and its presence was revealed by the ability to induce interference in fresh pieces of CAM.

This is a preliminary report showing that chorioallantoic fluid (CAF) infected with Ehrlich's tumor cell-transfered active strain NWS has the ability to suppress virus growth like interferon.

For the assay of the interfering activity, small pieces of CAM of 14-day old embryos with shell and Earle's solution with penicillin and streptomycin, as the suspending medium, were used. Virus in infected CAF was removed by repeated cycles of ultracentrifugation at 20,000 rpm. for 30 minutes and adsorption onto thrice washed chicken red blood cells (RBC). (cf. Table 1.)

Table 1.



Pieces of membrane were shaken at 37°C. for 20 hours with samples diluted with Earle's solution to incorporate the active substance. Then hemagglutination in the suspending medium was measured. No sample showed any hemagglutination.

After washing the pieces of membrane once with Earle's solution, 64 HAU of PR8 strain virus was added as a challenge virus. After allowing time for adsorption, the pieces of membrane were washed three times with Earle's solution

to remove non-adsorbed virus. They were placed in fresh test tubes containing 2 ml. of Earle's solution. After 48 hours incubation at 37°C, the hemagglutination titer of the suspending media in each tube was tested.

As controls the following were used: i) Earle's solution, ii) interferon produced by the action of heat-inactivated PR8 on CAM, iii) an interferon control i. e. Earle's solution incubated with CAM, iv) PR8-infected CAF treated by ultra-centrifugation and adsorption with RBC to eliminate the virus.

Table 2.

Exp. groups	mean $\log_2$ HA titers
Earle's sol. as control	6.6
interferon control	6.7
interferon	5.0
treated PR8-infected CAF	4.5
treated NWS-infected CAF	3.3
NWS-infected CAF diluted to 10^{-3} in Earle's sol.	4.6
NWS-infected CAF diluted to 10^{-4} in Earle's sol.	5.6

The results are shown in Table 2. The value for each group represents the geometrical mean of the six tubes used. The results suggest that NWS-infected CAF interferes more strongly than PR8-infected CAF or interferon.

It was impossible to completely remove the virus from the infected CAF, the following experiment was made to ascertain that the interference of this material was not due to any virus remaining in the treated CAF. First treated NWS-CAF was tested for infectivity. Then fresh NWS-CAF was diluted to the same infective titer as that of the treated CAF. These two samples were tested for their interfering activity.

Table 3.

Exp. groups	mean $\log_2$ HA titers
Earle's sol. as control	4.4
treated NWS-infected CAF ($EID_{50}=10^{3.2}$/ml)	<0.58
NWS-infected CAF diluted to 10^{-4} in Earle's sol.	4.4
NWS-infected CAF diluted to 10^{-5} in Earle's sol.	5.4

Results in Table 3 show that the interfering activity of the treated NWS-CAF was not due only to residual virus particles.

A study was made of whether the interfering effect in NWS-CAF was due to

a soluble antigen. To fractionate the S-antigen, treated CAF was further centrifuged at 39,760 rpm. for 14 hours. The upper 2/3 of the supernatant and the sediment resuspended in the original volume of Earle's solution were tested for interfering activity. The latter fraction could be regarded as the S-antigen fraction.

Table 4.

Exp. groups	mean $\log_2$ HA titers
Earle's sol. as control	5.3
treated normal CAF	4.9
sediment of 39760 rpm. for 14 hours as S-antigen	4.7
supernatant of 39760 rpm. for 14 hours	2.3
treated NWS-infected CAF (undiluted)	2.1
treated NWS-infected CAF (diluted to 10^{-1} in Earle's sol.)	4.3
treated NWS-infected CAF (diluted to 10^{-2} in Earle's sol.)	5.3

Table 4 shows that the interfering activity of NWS-infected CAF was not due to S-antigen, but to a non-hemagglutinating, non-sedimentable substance in the supernatant fluid.

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