known cadmium-zinc antagonism, it is possible that dietary zinc, iron, and ascorbic acid are important factors in modifying the adverse effects of cadmium in human beings.

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## Homologous Viral Interference: Induction by RNA from Defective Particles of Vesicular Stomatitis Virus

Abstract. *The viral RNA isolated from the defective particles of vesicular stomatitis virus was capable of interfering with the replication of this virus in chick embryo cells. The data indicate that the interfering ability of the defective particles of this virus is due to their nucleic acid component.*

Vesicular stomatitis virus (VSV), a single-stranded RNA-containing virus, replicates in mammalian tissue culture cells and produces not only the bullet shaped and infectious B particles but also defective T particles (*1*). Both the B and T particles possess the same kind and number of polypeptides and antigens (*2*). However, the T particles contain viral RNA which is only one-third the size of the RNA contained in B particles (*3*). Viral RNA's isolated from B and T particles have been shown to sediment on sucrose density gradients at a rate of 40*S* and 19*S* respectively (*3, 4*). Interest in studying the noninfectious T particles arose since it has been shown that T particles can interfere specifically with the infection of cells with B particles (*5, 6*). The interference induced by the T particles in cells depends on the relative input ratio of B and T added to the cells (*6*). Also it has been shown that the interference caused by T particles is an intracellular phenomenon not mediated by interferon (*6*). The interfering constituent of the T particles is probably the viral RNA, since ultraviolet light can abolish the interfering ability of T particles (*6*). This report deals with experiments demonstrating that the viral RNA alone, either isolated from T particles or from VSV-infected cells, can interfere specifically with the replication of VSV virus.

Chick embryo fibroblasts were prepared and grown in Eagle's medium according to procedures described earlier (*7*). Vesicular stomatitis virus stocks (Indiana strain) were prepared according to the method of Huang, Greenawalt, and Wagner (*1*). Concentrated and partially purified suspensions of B and T particles were obtained from chick embryo cultures infected with VSV.

The method used for partially purifying the two types of particles was the same as that used by Huang, Greenawalt, and Wagner (*1*). The particles were purified by two successive centrifugations on sucrose density gradients. The plaque-forming ability of purified T particles was less than 0.5 percent of that of the B particles. Viral RNA was released from the purified virus particles with 0.2 percent sodium dodecyl sulfate and 0.1 percent pronase and sedimented on sucrose density gradient by methods described by Huang and Wagner (*3*). The optical density of the fractions from the sucrose density gradient was measured. The sedimentation coefficient of the RNA from B particles was 40*S* compared with 19*S* for RNA from T particles. The fractions containing the 40*S* VSV RNA or the 19*S* VSV RNA were pooled and precipitated with alcohol. The viral RNA was further purified on a column of cellulose (Whatman CF-11) according to the method described by Franklin (*8*). The single-stranded RNA eluting in the 15 percent ethanol buffer was collected and precipitated with ethanol. Viral RNA purified by the above method was used for the interference experiments. Viral RNA's were also isolated from VSV-infected cells and purified by the above method.

The uptake of single-stranded RNA by mammalian cells is known to be enhanced by diethylaminoethyl dextran (DEAE dextran) (*9*). Therefore DEAE dextran was used for enhancing the uptake of the viral RNA into chick embryo cells. The interference experiments were performed as follows. Monolayer cultures of chick embryo cells containing $3 \times 10^6$ cells per culture were washed twice with serum-free medium and then incubated at 37°C for 10 minutes with 1 ml of phosphate-buffered saline containing 80 μg of DEAE dextran (Sigma Chemical Co.).

The DEAE dextran was then removed and 0.5 ml of phosphate-buffered saline containing the viral RNA and 80 μg of DEAE dextran were added to cultures. Control cultures received 0.5 ml of phosphate-buffered saline containing DEAE dextran. After 60 minutes at 37°C with frequent agitation the cultures were washed three times and then infected with VSV or Sindbis virus at an input multiplicity of

Table 1. Effect of preincubation of chick embryo cells with RNA on infection with VSV. PFU, plaque-forming units.

| RNA used for incubation* | Incubation with ribonuclease | Virus yield (PFU/cell)† |
|---|---|---|
| None | No | 650 |
| 40*S* RNA from B particles | No | 615 |
| 40*S* RNA from B particles | Yes | 670 |
| 19*S* RNA from T particles | No | 52 |
| 19*S* RNA from T particles | Yes | 670 |
| 19*S* RNA from VSV-infected cells | No | 63 |
| 19*S* RNA from VSV-infected cells | Yes | 655 |
| Ribosomal RNA from chick embryo cells | No | 630 |

* Ten micrograms of RNA used per $3 \times 10^6$ cells. † Average of two separate experiments. Incubation with ribonuclease was done before exposing RNA to the cells. Samples of RNA in phosphate-buffered saline were incubated with ribonuclease (1 μg/ml) for 10 minutes at 37°C.

Table 2. Properties of interference induced by the RNA from T particles. PFU, plaque-forming units.

| Experimental system | Virus yield (PFU/cell)* |
|---|---|
| 1) Chick embryo cells preincubated with or without 19*S* VSV RNA and challenged with VSV | |
| a) 19*S* RNA, 10 $\mu$g per $3 \times 10^6$ cells | 63 |
| b) 19*S* RNA, 1 $\mu$g per $3 \times 10^6$ cells | 285 |
| c) 19*S* RNA, 0.1 $\mu$g per $3 \times 10^6$ cells | 606 |
| d) Chick embryo cells, no RNA | 725 |
| 2) Chick embryo cells incubated with actinomycin D, followed by 19*S* RNA and challenged with VSV† | 42 |
| Chick embryo cells incubated with actinomycin D, no RNA, challenged with VSV | 435 |
| 3) Chick embryo cells incubated with 19*S* RNA and challenged with Sindbis virus† | 2250 |
| Chick embryo cells, no RNA, challenged with Sindbis virus | 2335 |

* Average of two experiments. Cultures were incubated with actinomycin D (5 $\mu$g/ml) for 1 hour.
† Concentration of 19*S* RNA used was 10 $\mu$g per $3 \times 10^6$ cells.

one plaque-forming unit per cell. The challenge virus was allowed to attach to cells at 37°C for 30 minutes. The cultures were then washed three times with phosphate-buffered saline and subsequently incubated at 37°C with Eagle's medium. The cells and medium from the cultures were harvested at the end of 16 to 18 hours and then assayed for infectious virus on chick embryo cells.

Interference experiments were carried out with the RNA preparations obtained from the B and T particles. The results presented in Table 1 indicate that the RNA obtained from B particles did not possess any significant capacity to interfere with the replication of infectious VSV. However, incubation of cells with the RNA from T particles resulted in 92 percent reduction in the yield of infectious VSV as compared with that in control cells. Additionally it can be seen from the results that interference of VSV by 19*S* RNA was specific, since under identical conditions ribosomal RNA isolated from chick embryo cells possessed no interfering ability. Incubation of the RNA with ribonuclease prior to its exposure to cells destroyed the interfering capacity of the viral RNA. Also it can be seen that the 19*S* RNA isolated from the VSV-infected chick embryo cells possesses interfering ability similar to that of the RNA from T particles.

The results presented in Table 2 summarize some of the properties of the interference induced by the viral RNA from T particles. The degree of interference induced by the RNA was dependent on the amount of viral RNA used to incubate cells before challenge. Interferon is a mediator in many instances of viral interference. However, the interference induced by interferon in cells could be blocked by actinomycin D (*10*).

It can be seen that the interference induced by the viral RNA was not sensitive to actinomycin D, since the degree of interference was similar in cultures incubated with or without actinomycin D prior to the addition of the viral RNA. This finding and the following observation indicate that the interference induced by the RNA from T particles is probably not mediated by interferon. In contrast to the results obtained with VSV, the viral RNA from T particles had no effect on the replication of Sindbis virus, an arbovirus (Table 2). These results indicate that the interference induced by the viral RNA was homologous and the observed interference was not due to the induction of interferon or some general toxic effects of the viral RNA in chick embryo cells.

The results presented here provide direct proof that the component of the T particles responsible for its interfering ability is the nucleic acid. The properties of the interference induced by the viral RNA isolated from T particles are similar to those reported for the whole T particles (*6*). The results also explain the observed dependence of the interference on the ratio of B and T particles added to tissue culture cells since the degree of interference induced by the 19*S* viral RNA depended on the concentration of the RNA used. At present the precise mechanism of the interference phenomenon induced by T particles is not known. A phenomenon similar to that described here occurs during the in vitro synthesis of bacteriophage Q$\beta$ RNA (*4*). Fragments of Q$\beta$ RNA (molecular weight, $1.7 \times 10^5$) competed successfully with the intact Q$\beta$ RNA (molecular weight, $1.0 \times 10^6$) for replicase in the in vitro RNA synthesizing system (*4*). The Q$\beta$ RNA fragments had a higher affinity for the replicase than the intact Q$\beta$ RNA. The interference in the replication of intact Q$\beta$ RNA was visualized as a consequence of the capacity of viral RNA fragments to compete successfully in the step involving the recognition between the replicase and its usual template, the intact Q$\beta$ RNA (*4*). Thus it was hypothesized that highly selective interference with the replication of viral RNA can be brought about by compounds capable of neutralizing the recognition mechanism between the RNA replicase and its homologous template (*4*). Stampfer, Baltimore, and Huang proposed that the homologous interference induced by the defective VSV particles could be explained on the basis of the model described for Q$\beta$ viral RNA (*11*). They observed that during infection of Chinese hamster ovary cells with VSV there was a changing pattern of viral RNA synthesis in the infected cells. The concentration of the 19*S* viral RNA, the species contained in T particles, increased in the infected cells under conditions of interference. These results led to the hypothesis that the 19*S* viral RNA has a higher affinity for the viral RNA replicase than the 40*S* RNA from B particles and that 19*S* RNA can replicate itself and inhibit the replication of the 40*S* RNA. If this supposition is true it follows that the 19*S* viral RNA should directly interfere with the replication of VSV. The results presented here support the above hypothesis, since addition of 19*S* viral RNA to cells can establish the interference. Thus the intracellular concentration of 19*S* viral RNA in the infected cells may govern the degree of interference in cultures infected with VSV.

A significant result of the studies reported here is that the system, as employed here, can be utilized to investigate the interference phenomenon at a molecular level. The interference test using the viral RNA, rather than the T particles, could be used to understand the relationship between the structure of the viral RNA and its interfering ability.

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## Aldrin: Removal from Lake Water by Flocculent Bacteria

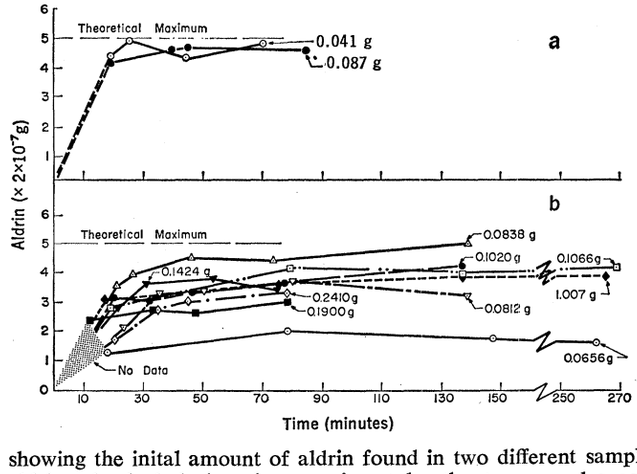

Abstract. *Floc-forming bacteria isolated from Lake Erie adsorb and concentrate aldrin from colloidal dispersion so that the settling of the bacterial flocs removes aldrin from the water phase. Contemporary sediments forming in Lake Erie contain aldrin and could adsorb more. The sediments consist of a conglomerate floc of bacteria, diatoms, and inorganic and detrital particles. Flocculent bacteria also adsorb microparticulates, and this adsorption capacity represents a mechanism for sediment formation and for the removal of suspended particles including aldrin from the water column.*


Many chlorinated hydrocarbon insecticides have been isolated from surface waters, usually in concentrations of less than 1 $\mu$g/liter or 1 part per billion (ppb). The deleterious effects of pesticide in water have been established (*1*). Our interest is in the fate of these chemicals in a water column and particularly in their adsorption to silt- and floc-forming bacteria which form contemporary sediment in lakes. Bacterial floc is an aggregation of cells which results in a macroscopic bacterial clump that settles from the liquid, thus leaving that medium less turbid. This type of growth appears to result from physical, chemical, and biological interactions when extracellular fibrillar polymers are synthesized by organisms (see *2*).

Our study of aerobic bacteria isolated from Lake Erie revealed that of 33 isolates tested in six different growth media 19 formed flocs in at least one medium, whereas ten formed flocs in two or more of the media. We report here a study of the ability of two of the floc-forming isolates to concentrate and accumulate the pesticide aldrin (*3*) from solution. One bacterium was an orange-red pigmented Gram-negative rod, tentatively identified as either a *Flavobacterium* or *Protaminobacter*. The other was a Gram-positive species of *Bacillus*.

Our experimental procedure was as follows: The test organisms were grown in a shake flask at ambient temperature (22° ± 2°C) in nutrient broth (8 g/liter, Difco), harvested by centrifugation, washed twice with distilled water, and resuspended in 25 ml of distilled water. Erlenmeyer flasks containing 50-ml suspensions of bacterial floc were then placed on a rotary shaker and 1 ml of aldrin dissolved in acetone was added to give a final aldrin concentration of $1 \times 10^{-6}$ g/ml or 1 part per million (ppm). After being shaken at 120 rev/min for the desired time period, the flasks were removed from the shaker and the floc was separated from the supernatant by centrifugation. The flocs were washed twice with distilled water and the washings were added to the original supernatant. The pesticide exposure time was calculated as that period between the addition of aldrin to the solution and the separation of the second washing from the bacterial floc. The floc and supernatant fractions were extracted separately with a mixture of heptane and acetone (3 : 1, by volume). The organic solvent fractions containing the aldrin were concentrated by evaporation and adjusted to a volume of 4 ml. Samples (2 $\mu$l each) were injected into a gas chromatograph (Aerograph model 200) fitted with an electron capture detector (*4*).

The total amount of aldrin adsorbed to bacterial floc as a function of time is plotted in Fig. 1. The theoretical maximum for aldrin adsorption calculated from a standard curve is 1 ppm. The recovery values for aldrin varied in individual experiments between 70 and 130 percent (0.7 to 1.3 ppm), with the variation possibly due either to adsorption on glassware (*5*) or to the varying sensitivity of the electron capture detector. Almost all of the aldrin ad-



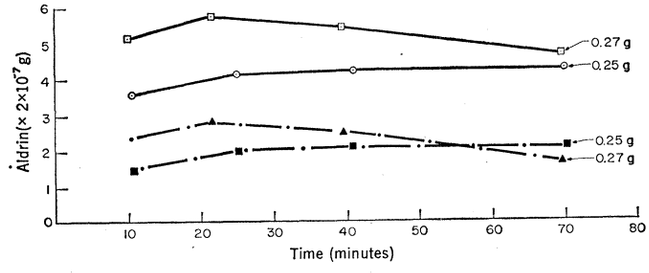




Fig. 1 (left). Curves showing adsorption of aldrin by both (a) Gram-positive bacterial floc and (b) Gram-negative bacterial floc as a function of time (solid lines). Numerals on each curve indicate the dry weight of bacterial floc used in each experiment. Broken and dashed lines indicate extrapolation from the first experimental point to zero time. Fig. 2 (right). Curves showing the inital amount of aldrin found in two different samples of contemporary sediment (silt) (broken lines) and the additional aldrin adsorbed during the experiment by the two samples as a function of time (solid lines).