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Effect of the Pesticide Carbaryl on Replication of Human and Simian Varicella Viruses

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Pretreatment of host cells with carbaryl delayed the early spread of simian and human varicella virus infections. Toward the end of the growth cycle there was an apparent enhancement of infection, since treated cultures showed more infectious centers than did untreated ones in which infectivity had reached maximum levels and then declined.

A recent report by Abrahamsen and Jerkofsky (1) described an enhancing effect of the pesticide carbaryl on the replication of varicella-zoster virus (VZV) in cell culture and suggested possible implications of an interaction between pesticides and VZV in the etiology of Reye's syndrome. In efforts to improve yields of a simian varicella-like virus (Delta herpesvirus [DHV]), we treated host cell cultures with a variety of agents reported to enhance herpesvirus replication, including carbaryl. Our findings indicate that rather than increasing the total amounts of human or simian varicella virus produced, carbaryl treatment of host cell cultures serves to delay the early spread of infection, with the result that cultures harvested at the end of the growth cycle show greater numbers of infected cells than do control cultures in which infected cells have reached maximum concentrations and then decreased.

The cell cultures employed were the L-645 line of human fetal diploid lung cells developed by J. H. Schieble of this laboratory and the BS-C-1 line of African green monkey kidney cells, obtained from H. E. Hopps, Bureau of Biologics, U.S. Food and Drug Administration, Washington, D.C. Cells were propagated by standard procedures (6). We used the VZV Batson strain isolated in this laboratory and DHV strain 592S (2), obtained from A. D. Felsenfeld, Delta Regional Primate Center, Covington, La. Cell-free virus was produced by sonic treatment of infected cultures, as previously described (8). The pesticide carbaryl was obtained through the courtesy of A. A. Puech and R. L. Baron, Union Carbide Corp., New York, N.Y. The actual concentration of carbaryl in our stock solution was determined by gas chromatography, kindly performed by W. A. Vance, Air and Industrial Hygiene Laboratory, California Department of Health Services. For virus growth studies, cell cultures in 2-oz (60-ml) prescription bottles were pretreated with carbaryl at a concentration of approximately 12 ppm for 18 to 20 h before they were inoculated with virus. This concentration of carbaryl was similar to the 18 ppm which Abrahamsen and Jerkofsky (1) considered to give maximal enhancement of VZV replication. Untreated cultures of the same lot served as controls. After the cell monolayers were washed with Hanks balanced salt solution, maintenance medium consisting of Eagle minimal essential medium with 5% fetal bovine serum was added to the cultures; they were then inoculated with either trypsin-dispersed, virus-infected cells (8) or with cell-free virus. Cultures were incubated at 36°C, and at daily or longer intervals after infection, duplicate infected cultures of carbaryl-treated and untreated cells were harvested. The culture fluids were removed and saved, and the cells were dispersed with trypsin and suspended in the culture fluids. The cell suspensions were assayed for infectious centers by plaquing (7) VZV in human fetal diploid lung cells and DHV in BS-C-1 cells. The data shown in the growth curves are based on average counts of infectious centers in duplicate cultures harvested at each time interval.

Figure 1 shows results of two representative experiments with different cell-free preparations of DHV. In both experiments, the spread of virus infection was delayed during the first part of the growth cycle, and toward the end of the cycles, when untreated cultures had attained maximum numbers of infectious centers and infectivity titers were declining, there were greater numbers of infectious centers in carbaryl-treated cultures than in control cultures. However, total numbers of infectious centers produced over the course of the growth cycle were similar in untreated and treated cultures. Similar results were obtained with VZV by using

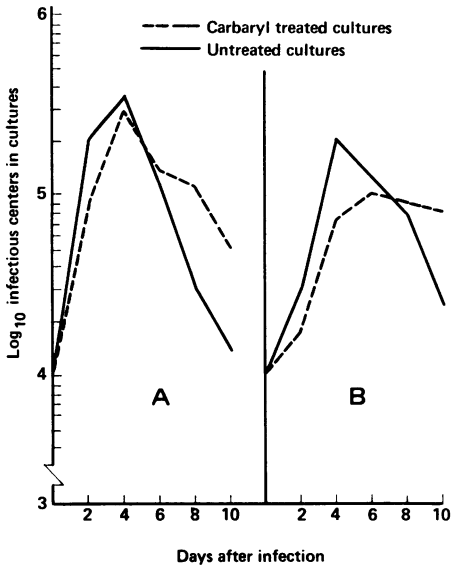

FIG. 1. Effect of carbaryl on replication of DHV in BS-C-1 cells. Two experiments were done with different cell-free virus preparations as inocula. Results are based on the average counts of infectious centers in duplicate cultures harvested at each time interval.

either trypsin-dispersed infected cells or cell-free virus as inocula (Fig. 2).

In other experiments (data not shown), it was found that pretreatment of BS-C-1 cells with carbaryl had no effect on the rate of viral attachment to the cells, and plaque counts of DHV in carbaryl-treated cultures were the same as, or slightly lower than, those in untreated control cultures.

Pretreatment of host cells with 10% dimethyl sulfoxide for 5 min followed by washing and 24 h of incubation before inoculation was seen to have an effect on the growth cycles of VZV and DHV similar to that caused by pretreatment with carbaryl (Fig. 3). Dimethyl sulfoxide is known to cause reversible depression of growth and inhibition of protein synthesis in cultured cells (3, 5), and it would appear that there is a delay of several days before the cultures return to a metabolic state in which they are as susceptible as untreated cultures to replication of varicella viruses.

Carbaryl has been shown to bind strongly to cell components, including proteins (4), and would also appear to exert effects on cellular metabolism which must be reversed over several days of incubation in order for the cultures to regain full susceptibility to VZV and DHV replication. At the intervals at which cultures were harvested for infectivity assays, carbaryl-treated and untreated cultures contained comparable numbers of total cells.

Since varicella viruses are so strongly cell associated and spread by cell-to-cell transfer, it would be expected that cell culture infection is markedly affected by suboptimal physiological conditions of the host cells. Our results illustrate that in investigating the ability of agents to enhance replication of varicella viruses, it is important to assay for infectivity throughout the growth cycle. Even with viruses which are not so strongly cell associated as varicella virus, it is important to assay infectivity throughout the growth cycle before concluding that a particular treatment has affected total viral replication, rather than merely altering the kinetics of replication.

It may be that the enhancement of VZV replication observed by Abrahamsen and Jerkofsky (1) was an apparent effect, seen at a point in the growth cycle at which infectivity had attained maximum levels and declined in untreated cultures, whereas cells in carbaryl-treated cultures which had been spared from early infection had finally become infected. In light of our findings, a proposed VZV-enhancing effect of the pesticide carbaryl and its possible implications in the etiology of Reye's syndrome might be reevaluated.

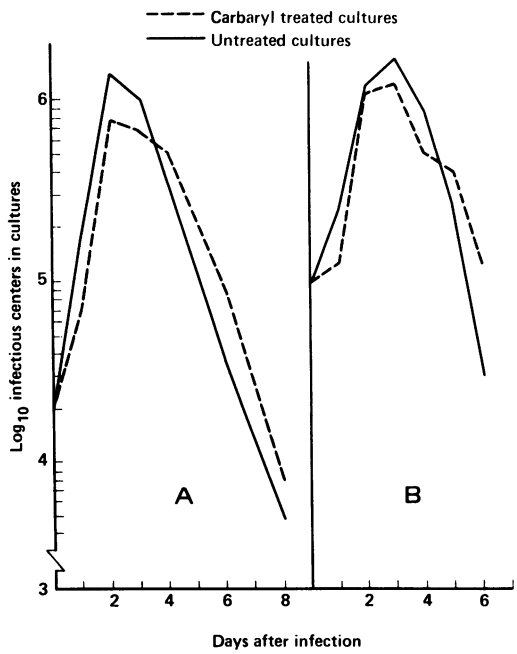

FIG. 2. Effect of carbaryl on replication of VZV in human fetal diploid lung cells. (A) Trypsin-dispersed infected cells used as an inoculum. (B) Cell-free virus used as an inoculum. Results are based on the average counts of infectious centers in duplicate cultures harvested at each time interval.

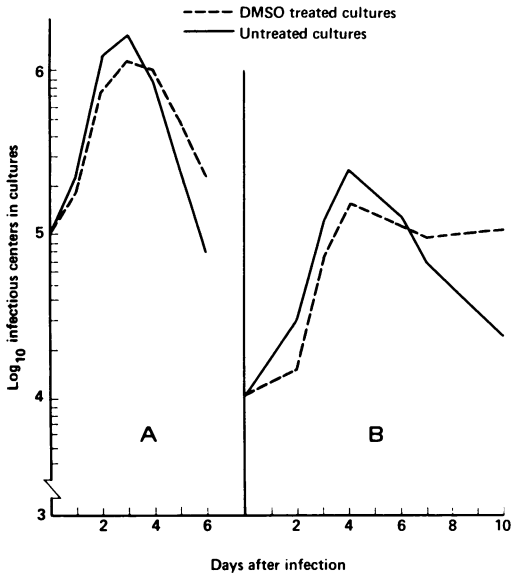

FIG. 3. Effect of dimethyl sulfoxide (DMSO) on replication of cell-free VZV in human fetal diploid lung cells (A) and cell-free DHV in BS-C-1 cells (B). Results are based on the average counts of infectious centers in duplicate cultures harvested at each time interval.

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