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# Inhibition of Herpesvirus Replication by Marine Algae Extracts

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Extracts from two species of marine red algae, *Cryptosyphonia woodii* and *Farlowia mollis*, specifically inhibited herpes simplex virus replication in vitro.

Studies on marine flora and fauna as sources of antiviral agents have not been extensive (1, 5) despite indications that such sources were potentially useful (2, 4, 7). This preliminary report describes the inhibition of types 1 and 2 herpes simplex virus (HSV-1 and HSV-2) replication in cell monolayers pretreated with extracts derived from two related species of *Rhodophyta*, *Cryptosyphonia woodii* and *Farlowia mollis*.

Algae were collected at Duxbury Reef, Bolinas, Calif., identified by species, and stored at −70 C. Frozen samples were combined with citrate-phosphate buffer (3) at pH 7.0 (20%, wet wt/vol), homogenized in a Waring blender, and then incubated at 4 C overnight.

Antiviral activity was assessed as follows. Clear extracts at a final 1% concentration (vol/vol) in Leibovitz L-15 medium supplemented with 2% fetal calf serum were added to human embryonic lung, Vero monkey kidney, or hamster kidney cell cultures at 2 or 24 h prior to inoculation with serially diluted virus. The criterion for antiviral activity was a reduction in virus infectivity between treated and control monolayers of at least 2 $\log_{10}$ (6). This degree of inhibition was observed only with HSV-1 and HSV-2. Eleven other viruses tested were not inhibited. These included coxsackie B5, echo-6, polio 2, rhino 2, respiratory syncytial, adeno 7, vaccinia, vesicular stomatitis, Japanese B encephalitis, Western equine encephalitis, and Sindbis viruses.

Portions of algal homogenates were combined with equal volumes of chemicals listed in Table 1 and held at 4 C for 24 h. Samples of clear aqueous phase were dialyzed against citrate-phosphate buffer or water or washed three times with ethyl ether prior to testing. The response of the virus inhibitor to the chemicals used indicated that the inhibitor was stable to shifts in pH, different osmotic pressures, salting out, and treatment with lipophilic solvents. The antiviral material also was nondialyzable and retained activity after being heated at 100 C for 20 min. Passage of active extracts through a Sephadex G-50 column gave fractions that indicated an association of antiviral activity with material of molecular weights of 10,000 or greater. These findings suggest that the active substance contains polysaccharides. Studies on its chemical composition have been initiated.

The mode of action of the inhibitor has not been determined as yet, although direct inactivation of virus and the induction of interferon synthesis have been ruled out as possible mechanisms. The inhibitor for HSV could be removed from cell monolayers by washing, even after some hours of contact at 37 C prior to virus inoculation. This suggests that the inhibitor may coat or block cell receptor sites required for herpesvirus infection. If such blockage does

TABLE 1. *Fate of herpesvirus inhibitor from C. woodii and F. mollis homogenates after treatment with selected chemicals*

| Activity retained[a] | Activity not retained |
|---|---|
| HCl (1 M) | Methanol[b] |
| Acetate buffer (0.1 M, pH 5.0) | Ethanol[b] |
| NaOH (1 M) | Acetone[b] |
| Glycine-NaOH buffer (0.1 M, pH 9.5) | Phenol (water-saturated) |
| NaCl (4 M) | Boiling acetic acid (0.2 M/20 min) |
| Distilled water | |
| Ammonium sulfate (2 to 4 M) | |
| *n*-Butanol | |
| Genetron 113 | |
| Chloroform | |
| Ethyl ether | |

[a] Clear aqueous phase, after chemical treatment and processing as described in text, inhibited both type 1 and type 2 HSV replication in vitro by at least 2 $\log_{10}$. Control HSV-1 and HSV-2 titered $10^5$ and $10^6$ mean tissue culture infective doses per ml, respectively.

[b] Activity found in precipitate.

occur, the reason for its specificity is not known. The prevalence of the inhibitor in marine algae also remains to be determined. Some uniqueness is substantiated by negative results from tests on some 25 other algal species.

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