glomerata, A. luchuensis, P. citrinum, Paecilomyces fusisporus, Sclerotium sp. and *A. sydowi* increased in their % occurrence in some of the metabolites of test fungi.

GARRETT[3] reported that the ability of tolerance to the toxin of metabolites by other organisms is directly related to the competitive saprophytic ability. DIX[1] reported that some frequently occurring fungi in rhizosphere succumbed to antagonism and did not appear on the root surface. Results in the present study demonstrate that the pioneer colonizers as well as secondary and late colonizers of rhizoplane, which have more tolerance capacity to the metabolites produced by the coexisting fungi, have considerable effect on the biological equilibrium of the rhizosphere. Thus the quantity of selective antagonist influences the colonization of root surface and the rhizosphere if not entirely, then to a great degree.

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[4] Grateful thanks are due to Prof. R. MISRA, Head of the Botany Department, for facilities and Dr. R. Y. ROY for suggestions.

Effect of Actinomycin D on the Multiplication of the Infectious Pancreatic Necrosis Virus of Trout

Actinomycin D, which blocks all DNA-directed RNA synthesis, inhibits the replication of DNA viruses. In general, RNA viruses are not effected by concentrations of actinomycin which inhibit cellular RNA synthesis. The antibiotic does, however, have an inhibitory effect on some RNA viruses. The growth of some strains of poliovirus under special culture conditions has been restricted with actinomycin D[1,2]. Also, the multiplication of some myxoviruses, including influenza virus, appears to be inhibited by relatively low concentrations of actinomycin added at early times after infection but not at later times[3-6]. The inhibition of reovirus replication by actinomycin appears to be concentration dependent. Reovirus type 3 replication in L cells is inhibited by 1–2 μg actinomycin per ml[7] and virus formation remains sensitive to inhibition throughout most of the replicative cycle[8]. However, lower concentrations of actinomycin (0.5 μg/ml), which reduce cell RNA synthesis by more than 90%, apparently have no inhibitory effect on reovirus replication[9,10].

Although initially designated a picornavirus[11-14], the infectious pancreatic necrosis (IPN) virus of trout has recently been found to resemble members of the reovirus group in size and morphology[15,16]. Preliminary evidence from studies in our laboratory indicates that the RNA of IPN virus is not double-stranded and the virus is, therefore not a reovirus; however, IPN virus was found to have an effect on macromolecule synthesis in the infected cell that is remarkably similar to that reported for the reoviruses (upublished data). Only one report of the effect of actinomycin D on IPN virus has appeared: MALSBERGER and CERINI[12] reported that actinomycin D, at a concentration of 2.0 μg/ml, resulted in a greater than 99% inhibition of IPN virus in rainbow trout gonad cell cultures. The report presented here describes studies to determine if, among other similarities, IPN virus resembles reovirus in its sensitivity to actinomycin D.

Monolayer cultures of the rainbow trout gonad (RTG-2) cell line[17], propagated at 22 °C, were employed as the host system, with the maintenance medium being Eagle's minimum essential medium with Earle's balanced salts solution and supplemented with 2% fetal calf serum. Actinomycin D (Calbiochem, Los Angeles, Calif. 90054) was stored in amber vials as an aqueous stock (200 μg/ml) from which the desired concentration was made in maintenance medium.

The same stock of RTG-2 propagated IPN virus (Dry Mills strain) was used throughout this work. Virus activity was assayed by infectivity titrations of supernatant and cell fractions sonicated for 1 min at 20 kc/sec (Sonic Dismembrator, Quigley-Rochester, Rochester, N.Y.). Infectivity titrations were made in Micro-Test II tissue culture plates (Falcon Plastics, Los Angeles, California) using a micro-titration technique. Infectivity titers are expressed in $TCID_{50}$'s as calculated by the method of REED and MUENCH[18].

The effect of various concentrations of actinomycin D on IPN virus multiplication is summarized in Table I. As shown, the production of infective IPN virus in RTG-2 cells was inhibited by more than 99% in the presence of all concentrations of actinomycin tested. Additional experiments demonstrated that the inhibition of IPN virus does not require continous exposure to actinomycin. Similar degrees of inhibition of the virus were observed in cultures which had been pretreated with actinomycin 1 or 2 h prior to infection, washed free of residual antibiotic, and incubated in normal maintenance medium.

The results presented in Table II indicate that the inhibitory action of actinomycin D involves a step very early in the replicative process of IPN virus. Pretreatment

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Table I. Effect of different concentrations of actinomycin *D* on the replication of infectious pancreatic necrosis (IPN) virus[a]

Concentration of actinomycin D (μg/ml)	Virus infectivity titer[b]
0.0	7.8
0.2	5.0
0.5	4.7
1.0	4.5
2.0	5.0

[a] Duplicate cultures of RTG-2 cells were pretreated with maintenance medium containing the designated concentrations of actinomycin D, 2 h prior to infection. Immediately before infection, the cultures were washed free of residual actinomycin with 3 changes of fresh maintenance medium. All cultures were then infected with approximately 100 $TCID_{50}$'s of virus per cell. Following a 1 h adsorption period at 22 °C, unadsorbed virus was removed by washing the cell sheet 3 times with Earles balanced salts solution. All cultures were then reincubated at 22 °C in maintenance medium containing the appropriate concentration of actinomycin D. The experiment was terminated at 24 h after infection. [b] Log_{10} of the number of $TCID_{50}$'s per 1.0 ml.

Table II. Effect of the time of addition of actinomycin *D* on the replication of infectious pancreatic necrosis (IPN) virus[a]

Actinomycin addition[b] (h)		Virus infectivity titer[c]
–[d]		7.8
1	Pre-infection	5.3
0		5.1
1	Post-infection	6.7
2	Post-infection	7.9
3	Post-infection	7.3
4	Post-infection	7.3
5	Post-infection	7.5
6	Post-infection	7.9
9	Post-infection	7.8
11	Post-infection	7.3

[a] All cultures were infected at time 0 with approximately 100 $TCID_{50}$'s of virus per cell as in Table I. Following virus adsorption the cultures were incubated in normal maintenance medium. The experiment was terminated 24 h after infection. [b] At the designated intervals, the normal maintenance medium on all cultures was replaced with maintenance medium containing actinomycin D (2.0 μg/ml). In the case of cultures treated with actinomycin prior to infection, maintenance medium containing the antibiotic was added immediately following the virus adsorption. [c] Log_{10} of the number of $TCID_{50}$'s per 1.0 ml. [d] Actinomycin D omitted.

of cells with actinomycin or the addition of the antibiotic at the time of infection resulted in a greater than 99% inhibition in the production of infective virus. A significant, but not as great, inhibition of IPN virus production was also observed when actinomycin was added within 1 h after infection. However, exposure to the antibiotic at 2 h after infection or later had no effect on the replication of IPN virus.

Microscopic examination of uninfected RTG-2 cultures treated with actinomycin D (2.0 μg/ml) revealed cells noticeably more rounded and granular than those of untreated controls. This cytotoxicity of actinomycin for RTG-2 cells was first apparent approximately 10–12 h after exposure to the antibiotic. The extent of cytological damage increased with time until 24 h after exposure, when cell damage and cell loss was extensive. The degree of cytotoxicity observed was less severe when concentrations of 0.2–0.5 μg actinomycin/ml were employed. The results presented in Table II indicate that the inhibition of IPN virus by actinomycin D is not an indirect result of the cytotoxicity of the antibiotic for RTG-2 cells. In cells exposed to actinomycin C from 2 h after infection until the experiment was terminated, IPN virus replicated to titers equivalent to those found in similarly infected untreated cells.

Although IPN virus resembles members of the reovirus group in size and morphology[15,16] and its effect on macromolecule synthesis in the infected cell (unpublished data), the results reported here indicate a sensitivity of the virus for actinomycin D which differs from that reported for the reoviruses. Instead, the pattern of inhibition of IPN virus by actinomycin D appears to parallel in many ways that described for the myxoviruses and poliovirus[1–6].

The mechanism by which actinomycin D inhibits the multiplication of IPN virus is not known. Assuming that IPN replicates in a manner similar to other single-stranded RNA viruses, an effect of actinomycin on the structure or activity of the replicative form (RF) is a possible mechanism. Alternatively, actinomycin may interfere with a constitutive host function required for virus multiplication. In any event, the actinomycin sensitive step in the replication of IPN virus occurs early in the infection process.

Résumé. La croissance du virus de la nécrose infectieuse pancréatique dans les cultures des cellules de la truite est arrêtée dans une proportion de plus de 99% par l'actinomycine D, à toutes les concentrations essayées. La réaction sensible à l'actinomycine a lieu dans les premières phases du processus infectieux.

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A Virus of a New Type Pathogenic to Crustacea

Very few virus diseases have been observed in marine invertebrates. In crustacea the only virus described is that discovered by Vago[1] in *Macropipus depurator* L. We have recently observed a new virus disease in *M. depurator* populations of the French Mediterranean coast. The diease is characterized by a weakening of the animals and a rapid mortality without obvious symptom.

From the haemolymph and from suspensions of different tissues of diseased crabs, an ovoid, often elongated, virus has been isolated. The virion, 150–300 nm in length, is surrounded by an envelope of composedsub-units arrang-

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