THE INHIBITION OF AN RNA BACTERIOPHAGE BY STREPTOMYCIN, USING HOST BACTERIA RESISTANT TO THE ANTIBIOTIC*

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In most studies on the effect of antibiotics on virus growth, the host cells used have been sensitive to the inhibitory agent, so that a specific antiviral effect has not been achieved. I should like to report studies on the inhibition by streptomycin of an RNA bacteriophage using Escherichia coli host cells that are completely resistant to the concentrations of antibiotic used. It will also be shown that streptomycin has no effect on a variety of DNA bacteriophages of E. coli under the same conditions.

E. coli strains 3000 and K10 #7 were obtained from Dr. W. A. Konetzka of this department. Both are hosts for an RNA bacteriophage, MS-2 (Davis et al., 1961), also obtained from Dr. Konetzka. Strain 3000 is also a host for a variety of E. coli DNA phages. Mutants were isolated resistant to 1000 μg/ml of streptomycin. These mutants grew at a normal rate in both the presence or absence of this concentration of streptomycin, and no reduction in growth rate occurred if the antibiotic was added to exponentially growing cells. Using ditritiostreptomycin (a gift of Dr. Charles Hurwitz), it was shown that in the primary phase of streptomycin binding (Plotz et al., 1961), the resistant mutant bound about 5×10^5 molecules of antibiotic per cell, while the

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sensitive parent bound 1×10^6 molecules per cell, when the initial streptomycin concentration was 50 μg/ml. In the secondary phase of streptomycin binding, the resistant strain bound very little additional antibiotic, whereas in the sensitive strain the amount bound rose to 2×10^7 molecules per cell. In the work to follow, unless stated otherwise, the resistant mutant was used with a concentration of antibiotic of 1000 μg/ml.

In initial experiments, a variety of bacteriophages were titred by the soft agar method (Adams, 1959) in parallel on streptomycin and plain agar plates, using strain 3000. The following bacteriophages gave essentially the same number of plaques on both plates: T1, T2, T3, T5, T7 and Phi X 174. Note that the last named has single-stranded DNA, whereas the others have double-stranded DNA. Under the same conditions, the RNA phage MS-2 was completely inhibited and no plaques were formed even when 10^9 plaque-forming units per plate were used. Streptomycin gave complete inhibition at 250 μg/ml and partial inhibition down to 50 μg/ml.

Streptomycin has only a slight effect on free MS-2, since after two hours incubation at 37° C with 1000 μg/ml of antibiotic, about 65% of the initial titre remained. This slight inactivation of free phage cannot explain the fact that streptomycin is able to prevent the replication of at least 10^9 plaque-forming units on plates. Streptomycin also has no effect on the rate of adsorption of MS-2 to the host bacteria.

To study the fate of intracellular phage, cells were grown and infected in the presence of streptomycin, and free phage removed by membrane filtration. If these infected cells were kept in the presence of streptomycin, the phage titre slowly dropped, apparently due to irreversible inactivation. If the streptomycin was removed immediately after filtration, replication occurred, but only after a 10-30 minute lag, and the final phage yield was reduced over the control. However, if the cells were infected in the absence of streptomycin, and the antibiotic was added 5 to 10 minutes later, it had no effect on the

lytic cycle, and a normal burst occurred. Since the latent period is about 15 minutes (Loeb and Zinder, 1961), this means that streptomycin is able to inhibit only when present very early in the replication cycle.

It thus appears that streptomycin is able to inhibit when it is present at the time of adsorption and injection, but not when added shortly after injection has occurred. Zinder (1962) has shown that RNAse will not inactivate free RNA phage, or intracellular phage, but is able to inactivate when it is present at the time of adsorption. He has postulated that there is a transient condition immediately after adsorption when the phage becomes RNAse sensitive. The streptomycin effect may be explained in a similar way. It may be postulated that after adsorption of the phage, the RNA is liberated from the protein coat in a location where it is accessible to streptomycin, possibly at the periphery of the cell. If the streptomycin is added at this time, it combines with the RNA and inhibits replication. After this initial existence in a streptomycin-accessible site, the RNA moves to another site where streptomycin cannot reach it. If streptomycin is added at this latter stage, replication can occur normally.

If such an explanation is correct, streptomycin might be expected to affect any virus which existed transiently in an accessible site. I have also found that streptomycin is able to prevent the replication of several viruses which attack Streptococcus faecium, and at least one of these has been shown to be a DNA virus. This would imply that streptomycin-sensitive bacteriophages do not possess any specific type of nucleic acid, but probably occupy a site during the early stages after adsorption which is accessible to streptomycin even in streptomycin-resistant bacteria. This hypothesis does not make any assumptions about the differences between streptomycin-resistant and streptomycin-sensitive bacteria. The effects noted here might also be found with sensitive bacteria, but it would not be possible then to state that the effect was phage-specific.

The implication of the present work for antiviral chemotherapy seems clear. Because the bacteria are resistant to the antibiotic, streptomycin

is acting as a specific antiviral agent. Apparently the antibiotic has no effect on either RNA or DNA animal viruses, since it is usually added to cell cultures in the cultivation of these viruses. Animal cells may be impermeable to streptomycin, but if the antibiotic could be gotten into these cells, inhibition of intracellular virus replication might occur.

REFERENCES

ADAMS, M. H., Bacteriophages, Interscience Publishers, Inc., N.Y., (1959).
DAVIS, J. E., STRAUSS, J. H. and SINSHEIMER, R. L., Science, 134, 1427 (1961).
LOEB, T. and ZINDER, N. D., Proc. Nat. Acad. Sci. 47, 282 (1961).
PLOTZ, P. H., DUBIN, D. T. and DAVIS, B. D., Nature, 191, 1324 (1961).
ZINDER, N. D., Eli Lilly Award Address, American Society for Microbiology, Kansas City, Mo., May 9, 1962.