VARIANTS OF *SHIGELLA FLEXNERI* SENSITIVE TO NEOCARZINOSTATIN AND ACTINOMYCIN D

Sir:

The antimicrobial activity of neocarzinostatin, an antitumor protein, was reported to be limited to a number of Gram-positive organisms, most sensitive of which was *Sarcina lutea*[1]. Recently, in a study of the effect of neocarzinostatin on the transfer of R-episome from the *Escherichia* to the *Shigella*, we observed that two strains of *Shigella flexneri* were more sensitive than *S. lutea* to neocarzinostatin. Furthermore, these two neocarzinostatin sensitive *Shigella* strains are also found to be inhibited by actinomycin D. These strains were designated as C11 and C27 and both of them were identified as *Shigella flexneri* 2a by means of biological and serological procedures. Except for these two strains, the more than 40 other *Shigella* strains so far examined were not susceptible to these two antibiotics.

The minimal inhibitory concentration of neocarzinostatin against *S. lutea* by both the agar dilution and standard cup methods was 0.05 mcg/ml. In broth dilution tests, the two *Shigella* strains were inhibited by as little as 0.02 mcg/ml of neocarzinostatin (Fig. 1). There was not a sharp decline of the turbidity of the culture, showing no possible induction of *Shigella* phage, as was the case with *Bacillus subtilis* treated with this antibiotic[2].

These strains were sensitive to 1 mcg/ml of actinomycin D (Fig. 2). Other *Shigella* strains did not show any sign of sensitivity to 5 mcg/ml concentration of actinomycin D.

Neocarzinostatin inhibits the incorporation of ^{3}H-thymidine into DNA in strain C11 (Fig. 3). At levels above 0.05 mcg/ml the incorporation of the label into acid-insoluble fraction is stopped immediately. At 0.05 mcg/ml, incorporation of label was reduced more than 80 percent. At 0.005 mcg/ml, the inhibition was evident at 5 minutes but with increasing time there was less inhibition as with *S. lutea*[3] and HeLa cells. The treatment of the same C11 strain with 0.1 mcg/ml of actinomycin D led to a specific inhibition of the incorporation of ^{3}H-uridine into RNA as shown in Fig. 4.

Ten times this concentration (1 mcg/ml) was required for complete inhibition of the cell growth in the same medium.

The resistance of some enteric bacteria to some antibiotics many by explained by the inpermeability of the bacterial cells to the drugs. For instance, actinomycin D does not inhibit the RNA synthesis of *E. coli* either whole cells or spheroplasts[4]. *E. coli* which was treated with EDTA and loses some lipopolysaccharide components of the cell wall and becomes sensitive to actinomycin D[5] and neocarzinostatin[6].

Our observation indicate that variants of some of the enteric bacteria, normally insensitive to neocarzinostatin and actinomycin D, are found to be sensitive to these antibiotics. Biochemically, the both antibiotics have the same site of action in the variants. It is probable that the membrane structure may be

Fig. 1. Effect of neocarzinostatin on the growth of *Shigella flexneri* C11 and C27.

The overnight cultures of the bacteria in a Trypticase Soy Broth (TSB) were diluted in TBS, incubated for 3 hours and then diluted again in the same media to 5×10^6 cells/ml. Neocarzinostatin was added at the indicated concentrations (at time 0) and the turbidity at 620 mμ.

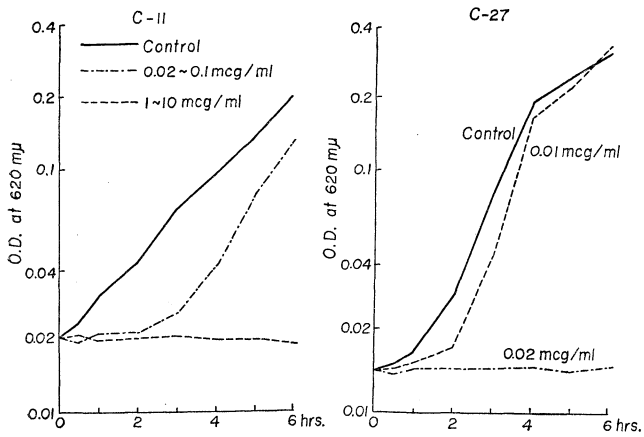


Fig. 2. Effect of actinomycin D on the growth of *Shigella flexneri* C11 and C27.

Conditions were the same as Fig. 1 except that actinomycin D was substituted for neocarzinostatin.

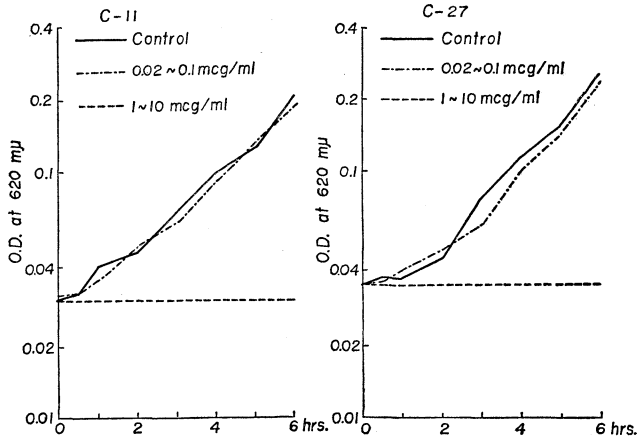


Fig. 3. Effect of neocarzinostatin on the uptake of ^{3}H-thymidine by *Shigella flexneri* C11.

5×10^{6} cells/ml of exponentially growing culture in TSB was treated with neocarzinostatin at various concentrations at time 0. At appropriate intervals, 0.05 ml of 2μc ^{3}H-thymidine (5.0 ci/mM) was added to 0.5 ml aliquots. The mixture was shaken for 5 minutes at 37°C. The labeling was terminated by chilling the mixture in an ice water bath followed by the addition of an equal amount of 10 % cold perchloric acid (PCA) together with 1 mg of carrier bovine serum albumin. The acid-insoluble precipitates were centrifuged and washed twice with 5 % cold PCA, the precipitates were dissolved in a 1 ml of 1 N NH_4OH, and an aliquot was taken into a vial contained in the Packard Tri-Carb liquid scintillation spectrometer with BRAY's scintillation fluid[8].

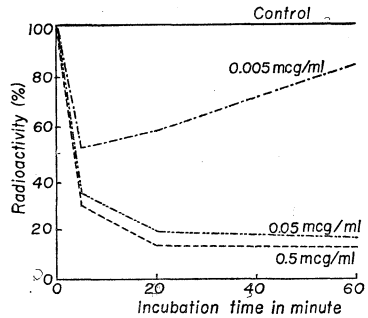
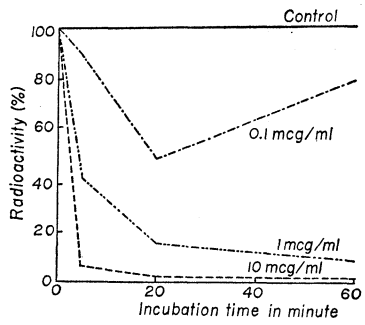


Fig. 4. Effect of actinomycin D on the uptake of ^{3}H-thymidine by *Shigella flexneri* C11.

Conditions were the same as Fig. 3 except that actinomycin D was substituted for neocarzinostatin and ^{3}H-uridine for ^{3}H-thymidine.



altered so that the drugs could panetrate into these variants. To date, the variants sensitive to actinomycin D are also sensitive to neocarzinostatin and *vice versa*[6].

Because of the extreme sensitivity of these organisms to neocarzinostatin they have been used in determining the distribution and excretion of neocarzinostatin in rats and mice[7].

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