Effect of Nitroacridine 3663 on Synthesis of Deoxyribonucleic Acid and Multiplication of T_2 Phage

It is known that the different acridine derivatives are able to become bound to isolated nucleic acids[1]. The compounds are more strongly bound by the highly polymerized deoxyribonucleic acid than by the ribonucleic acid[1]. The results of studying the interactions with deoxyribonucleic acid and the strong antibacterial effects of nitroacridine[2] suggest that these compounds may be used as virus chemotherapeutic agents.

The purpose of this work was to obtain information about the mode of action of nitroacridines. We used *Escherichia coli* strain *B* and phage T_2, employing the standard phage methods. The nitroacridine 3663 used was described by Schnitzer[2].

Bacteria were grown at 37° C. to a concentration of 2×10^8 per ml. in 2,500 ml. *M*-9 medium, sedimented and infected with 5 phage per bacterium in a non-nutrient adsorption medium[3]. The infected cells were then re-sedimented and transferred to a warm *M*-9 medium at 2×10^8 cells per ml. (zero time) which contained various concentrations of the compound.

The phage yields were measured at time zero and at other times from 1-ml. samples, diluted into a lysing medium[4] for intracellular bacteriophage determinations.

Immediately after infection and at various times later, samples of 500 ml. were withdrawn from the culture flask and quickly poured into 50 ml. of 3 *M* trichloroacetic acid. The fractions of deoxyribonucleic acid of the 500-ml. samples were obtained by a modified Schmidt–Thannhauser procedure[5]. Small aliquots of the trichloroacetic acid extracts containing deoxyribonucleic acid were analysed for deoxyribose by the diphenylamine method. The bulk of the extracts of deoxyribonucleic acid were evaporated to dryness and then hydrolysed[5]. The hydrolysates were chromatographed[6] and the amounts of the 5-hydroxymethylcytosine were determined spectrophotometrically.

The growth of the *E. coli B* was measured in *M*-9 medium by the colony plate count and by the nephelometric method. The compound was added in various concentrations to the culture when the growth was in the logarithmic phase.

As shown in Fig. 1, nitroacridine 3663 inhibits the synthesis of infective T_2 phage similarly to proflavine[4]; at the same time it has no effect on the multiplication of the host cell. The inhibition of the phage deoxyribonucleic acid occurs only at higher acridine concentrations. The degree of inhibition agrees entirely with the host cell.

It is thus seen that the effect of nitroacridine 3663 depends on the concentration of the compound. At very low concentrations (0·05–0·15 μgm./ml.) it inhibits the synthesis of T_2 phage without inhibiting the multiplication of the host cell. To account for this phenomenon it appears that during the vegetative phase of phage development the protein and deoxyribonucleic acid parts of phage are synthesized separately by the host cell[7]. The compound is bound more strongly by the structure-free phage deoxyribonucleic acid than by the structural nucleoproteins of the host. The deoxyribonucleic acid-acridine compound formed cannot be organized into an infective phage. In other words, this compound is a decoupling agent of the synthesis of phage nucleoprotein.

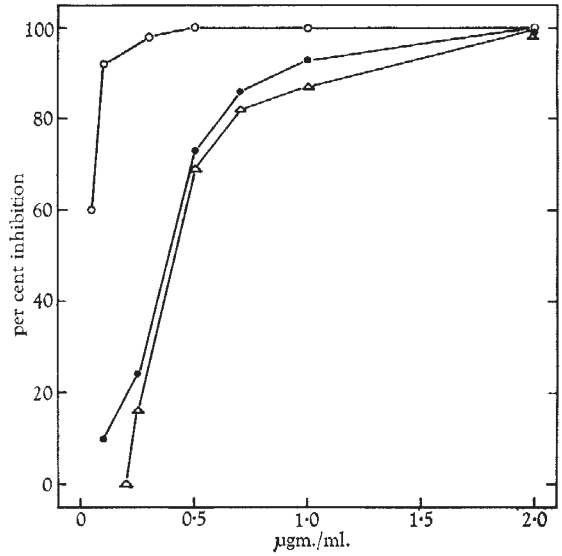

Fig. 1. Inhibitory effect of nitroacridine 3663 on —●—●—●— phage deoxyribonucleic acid synthesis (●); —○—○—○— multiplication of T_2 phage (○) and —△—△—△— multiplication of *E. coli B* (△), in per cent of untreated controls

At higher concentrations the compound inhibits the synthesis of phage deoxyribonucleic acid as well as the multiplication of *E. coli B*. The inhibitory effect is similar. From this observation it is concluded that the bacteriostatic effect of the compound is displayed by the inhibition of the synthesis of deoxyribonucleic acid.

Details of the relations of the structure and the effect of the different nitroacridines will be published elsewhere.

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A Simple Test for the Presence of Metals in Biological Materials

The presence of metals in highly purified protein preparations is of considerable interest. The paramount part played by metals, especially in enzyme reactions (see, for example, ref. 1), is well known. Besides the specific metals in the metalloproteins, most proteins are able to bind, more or less firmly, metals removed from the medium. The removal of metals from a protein solution may be an important preliminary step in a study of its properties and structure. Unfortunately, the available methods for the detection of metals involve the use of very expensive equipment, or are laborious, and require large quantities of protein.

A very simple, non-specific test was devised to demonstrate the presence of metals in protein