THE EFFECT OF MITOMYCIN C ON THE INDUCED SYNTHESIS OF PENICILLINASE IN STAPHYLOCOCCUS AUREUS

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Conflicting reports exist in the literature as to the mode of action of mitomycin C in bacteria. Kersten and co-workers (1963, 1964) suggest that the primary action of this antibiotic in E. coli is on RNA. They postulate that the observed degradation of DNA is brought about by destruction of an RNA-DNase inhibitor, a process dependent on the magnesium concentration. On the other hand, the results of Iyer and Szybalski (1963) with E. coli and B. subtilis and Matsumoto and Lark (1963) using E. coli would point to a primary action of mitomycin on DNA. Shiba et al. (1959) and Sekiguchi and Takagi (1960) found that mitomycin at low concentrations inhibits DNA synthesis in E. coli whereas the synthesis of protein and RNA continues normally for some time. It had previously been shown by Shiba et al. (1958) that mitomycin, at concentrations which completely inhibit DNA synthesis does not interfere with the induced synthesis of β-galactosidase in E. coli. We have observed that mitomycin inhibits the induced formation of penicillinase in Staphylococcus aureus. Investigations into the mechanism of this inhibition have been carried out in the belief that they might help resolve the above controversy. This work was presented in part to the Australian

Biochemical Society Meeting, Adelaide, January, 1965.

Materials and Methods. A readily inducible strain of S. aureus (phage type 80/81) was selected from several clinically available. Cells for induction were grown in nutrient broth at 37° with shaking and were harvested in the early logarithmic phase by centrifugation. Between 50 and 80 µg./ml. of cells were used in the induction medium. Cloxacillin (Orbenin or 3-o-chloro-phenyl-5-methyl-4-isoxazolyl penicillin), a gratuitous inducer for penicillinase, was a gift of Beecham Ltd., Brentford, U.K. Mitomycin C was obtained from Kyowa Hakko Co. Ltd., Tokyo. Uracil-2-C^{14} was obtained from the Radiochemical Centre, Amersham, and thymidine-H^3 from Schwarz Laboratories, Inc., Mount Vernon, N.Y. Radioactive compounds were diluted with the corresponding unlabelled material to give the required specific activities. Samples for the measurement of C^{14}-uracil and H^3-thymidine incorporation into nucleic acids were precipitated with ice-cold 5% (w/v) trichloroacetic acid, allowed to stand at 0° for 30 min., collected and washed on DA Millipore filters, dried and then counted in a Packard Tricarb liquid scintillation counter.

Results and Discussion. Figure 1 shows the effect of increasing concentrations of mitomycin (a) on the induced synthesis of penicillinase and (b) on DNA synthesis in S. aureus. It can be seen that both processes are inhibited by mitomycin, but the synthesis of DNA is approximately ten times more sensitive. Complete inhibition of DNA synthesis was not obtained under these conditions, and the reason for this appears to be due to the fact that an immediate inhibition is not achieved (see Fig. 2). The lowest concentration of

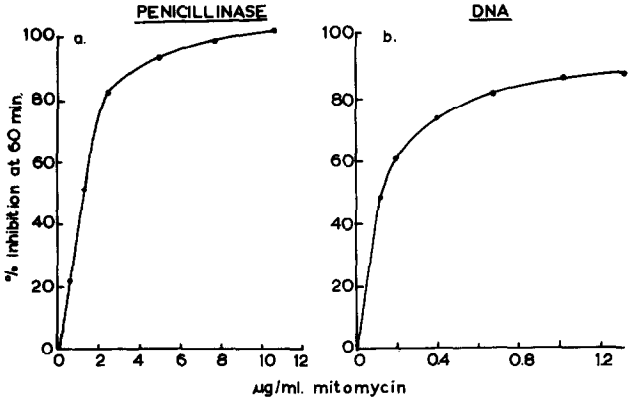

Fig. 1. Effect of increasing concentrations of mitomycin (a) on the induced synthesis of penicillinase and (b) on DNA synthesis. Experiments were carried out at 37° in a medium of the following composition: 0.1 mg./ml. glycine and the l-isomers of alanine, arginine, aspartic acid, glutamic acid, asparagine, glutamine, serine, leucine, isoleucine, valine, lysine, methionine, histidine, proline, tryptophane, phenylalanine, tyrosine and dl-threonine; 0.02M glucose; 2 µg./ml. nicotinamide; 0.2 µg./ml. thiamine; 1.0 mM mercapto-ethanol; 0.1 mM thymidine; 0.1 mM uracil; 0.1 mM KCl; 0.1 mM $MgSO_4$; 0.1 mM Na_2HPO_4; 0.017 M $NaHCO_3$; final pH 7.2. Cloxacillin (0.5 µg./ml.) and mitomycin were added at time 0. Fig. 1 a: Chloramphenicol (60 µg./ml.) was used to terminate the reaction and penicillinase was assayed manometrically (Henry and Housewright, 1947) at 37° using sodium penicillin G as substrate. Fig. 1 b: Thymidine-H^3 (sp. act. 8.3 µC./µmole) was used, and incorporated radioactivity determined as described at time 60 min. All experiments carried out in dim light.

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mitomycin giving near maximal inhibition of DNA synthesis was 1.3 µg./ml. and this caused about 50% inhibition of penicillinase production. This concentration was therefore used in subsequent experiments. A study of the inhibitions of the syntheses of DNA, RNA and penicillinase caused by this concentration of mitomycin, presented in Fig. 2 shows that (a) it takes from 15 to 20 min. for complete inhibition of DNA synthesis to take place; (b) penicillinase synthesis is inhibited only slightly during the first 40 min. of incubation, at which time however

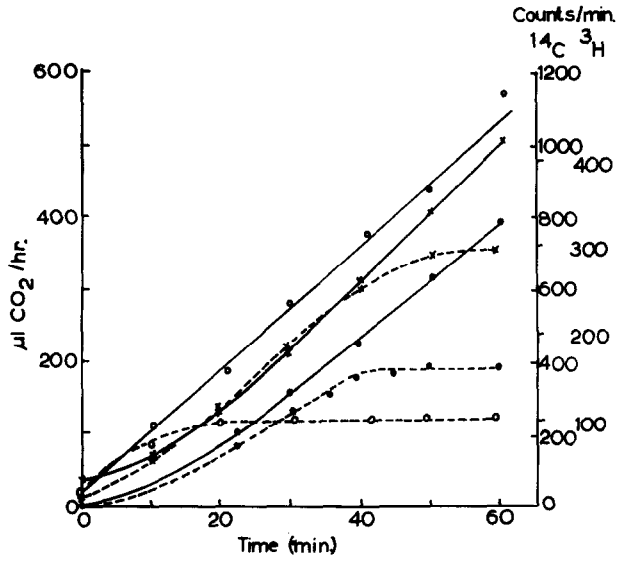

Fig. 2. Effect of mitomycin on the induced synthesis of penicillinase and the incorporation of uracil and thymidine. Conditions as in Fig. 1. Uracil-C^{14} (sp. act. 0.1 μC./μmole) and thymidine-H^3 (sp. act. 8.3 μC./μmole) were used. 1.0 ml. samples were removed at intervals and incorporated radioactivity determined as described. Penicillinase assays were carried out on 2.0 ml. samples as in Fig. 1. Controls (——), with 1.3 μg./ml. mitomycin (- - - -), penicillinase (●), uracil (×) and thymidine (o).

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further synthesis abruptly ceases; and (c) inhibition of RNA synthesis follows kinetics very similar to those of penicillinase formation. Under the above conditions, the incorporation of C^{14}-leucine into hot trichloroacetic acid-insoluble products followed the same time curve as that of penicillinase synthesis. When 0.67 μg./ml. mitomycin was used and penicillinase synthesis followed with time, again there was little inhibition initially and abrupt cessation took place 50 min. after the addition of the drug. Similar results to the above were obtained when the concentration of Mg^{++} was increased from 0.01 mM to 2.0 mM.

It is significant that the inhibition of penicillinase synthesis follows very closely that of uracil incorporation and the uracil incorporated appears to be metabolically stable, indicating (a) cessation of RNA synthesis including that of messenger RNA after 40 min. contact with mitomycin; and (b) stability of ribosomal RNA during the whole of the incubation period. The abrupt cessation of penicillinase synthesis would be a reflection of the cessation of the synthesis of penicillinase-specific messenger RNA which we have demonstrated in the cells with a half-life of less than one minute. We would expect a more gradual falling off in the rate of synthesis of penicillinase if mitomycin had acted on ribosomal RNA or SRNA as postulated by Kersten et al. (1963).

The results obtained by us are consistent with the independent proposals of Iyer and Szybalski (1963) and Matsumoto and Lark (1963), that the initial action of mitomycin is to cause covalent linking of the complementary strands of DNA at remote points along the chain. Under these conditions, we would expect the template activity of DNA to be affected earlier for DNA polymerase than for RNA polymerase. It is easy to conceive that one such cross link per DNA molecule would be sufficient to affect DNA polymerase, but RNA polymerase, which involves much shorter sections of template DNA would not be affected until cross-linking becomes more complete. In our own experiments it appears that it takes 40 min. before cross-linking is extensive enough to inhibit completely RNA polymerase and thus penicillinase synthesis.

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