Ces divers effects de la ribonucléase ne se manifestent pas si le site enzymatiquement actif est préalablement bloqué par carboxyméthylation d'une histidine suivant la méthode de BARNARD ET STEIN[2]. Il paraît donc probable que la ribonucléase agit sur la structure d'acides ribonucléiques intervenant dans la synthèse des protéines de phage et responsables de leur structure spécifique. L'altération de cette dernière expliquerait parfaitement l'absence de réaction entre les protéines anormales formées et les anticorps correspondant aux protéines normales. Les caractères chromatographiques des protéines phagiques libres synthétisées en présence de ribonucléase s'expliqueraient de même très aisément si nous admettions qu'elles appartiennent à de nombreux types aberrants alors que les protéines phagiques libres des cultures témoins ne peuvent appartenir qu'à un très petit nombre de types puisqu'elles sont sans doute essentiellement les précurseurs des particules mûres[3,4].

Un choix entre les diverses hypothèses qui pourraient être faites concernant le mécanisme de l'action de la ribonucléase sur la structure d'acides ribonucléiques intervenant dans la synthèse des protéines de phage paraît impossible actuellement.

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Bacteriocidal action of mitomycin C

The results of several investigations[1,2] have shown that the anti-tumour antibiotic, Mitomycin C, possesses antibacterial and radiomimetic properties. Moreover, in susceptible microorganisms it inhibits the biosynthesis of DNA[3]. The action of Mitomycin suggested a possible relationship to the phenomenon of "thymineless death[4]", a characteristic potentially useful for the selection of biochemical mutants in bacterial populations[5]. We have therefore examined its effect on several strains of *Escherichia coli*. The results show that Mitomycin C causes these bacteria to break down their DNA rapidly, acid-soluble products being formed.

A 12-h culture of *E. coli* (strain 15T$^-$), grown with shaking at 37°, was diluted into 5 vol. glycerol–ammonium-salts medium supplemented with thymidine (30 mg/l). The bacteria were harvested by centrifugation while in the logarithmic phase of growth, washed thrice with thymidine-free medium, and resuspended in medium containing [^{3}H]thymidine. Following 90-min incubation under conditions which prevented the occurrence of "thymineless death", the culture was once again centrifuged, the bacterial pellet washed free of radioactive thymidine, resuspended and incubated in fresh medium containing graded concentrations of Mitomycin C. After suitable intervals of time, the cultures were acidified with cold dilute $HClO_4$ and centrifuged. The supernatant solutions were neutralized with KOH, and the salts

Abbreviations: DNA, deoxyribonucleic acid.

which precipitated in the cold were removed by centrifugation. The radioactivity of the extracts was determined in a windowless gas-flow counter.

The results obtained in such an experiment are given in Table I. It is seen that cultures treated with increasing concentrations of Mitomycin C yielded a substantial proportion of their DNA in the form of acid-soluble degradation products. The accumulation of these substances was not associated with cell lysis, and occurred with cells which were actively synthesizing RNA and protein. In some experiments, up to 63 % of the DNA originally present became acid-soluble within 2 h. The presence of thymidine (or thymine) did not influence these effects of Mitomycin treatment.

TABLE I

APPEARANCE OF [^{3}H]THYMIDINE IN ACID-SOLUBLE FRACTION

Mitomycin (μg/ml)	*Extract (counts/min)**
Zero-time control	989
0	1,710
0.5	12,200
1.0	16,000
2.0	31,000
5.0	47,500
10.0	66,600
20.0	74,300

* Corrected for self-absorption. Samples incubated for 2.5 h, 37° (see text).

The rate of breakdown, which varied with bacterial population density as well as with the concentration of Mitomycin C, was linear with time for periods up to 2.5 h (Fig. 1). Chromatography of the extracts of such cultures has shown that about 95 % of the radioactivity was in thymine; the remainder was present as thymidine and a nucleotide which was not thymidine 5'-phosphate.

This action of Mitomycin C has been found to require the presence of Mg^{++} and to be inhibited by streptomycin, as in the breakdown of the DNA of superinfecting bacteriophages[7]. Following a brief exposure, the presence of the antibiotic is not required for a maximal rate of DNA breakdown to persist. Experiments have been performed to compare the rates of DNA synthesis and breakdown over a range of concentrations of Mitomycin C, these processes being measured respectively by uptake into and release from the acid-insoluble fraction of radioactive precursors or breakdown products. The resultant data are consistent with the assumption that the fraction of a bacterial population which is affected by Mitomycin and depolymerizing its DNA does not incorporate precursors into DNA, and, conversely, that the remaining fraction, which is capable of incorporating thymidine into DNA, does not release acid-soluble fragments into the medium. It should be noted that bacteria which have undergone "thymineless death" do not degrade their DNA, and that this effect of Mitomycin C is therefore not merely the result of inhibition of DNA synthesis.

Examination by phase[6] and electron microscopy of bacteria treated with Mitomycin has made possible the clear visualization of disintegrating nuclear bodies. These and other morphological, genetic, and biochemical findings form the subject of a full-length communication which is being submitted for publication to this journal. They suggest that the primary action of Mitomycin C leads to a scission of the DNA strand in susceptible bacteria; such DNA would no longer be capable of

replication, and the observed inhibition of synthesis follows. This formulation would appear to explain satisfactorily the irreversible bacteriocidal action of Mitomycin C.

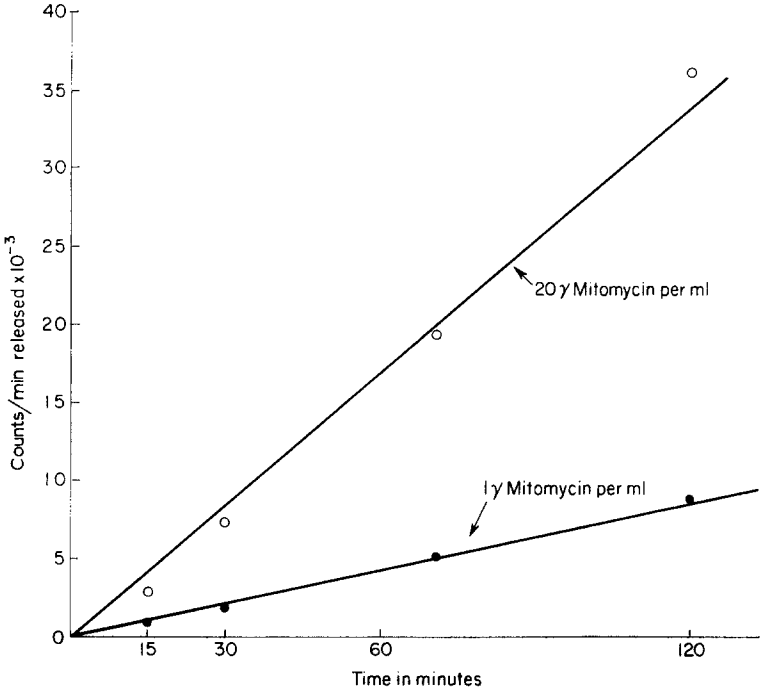

Fig. 1. Time course of appearance of [^{3}H]thymidine in acid-soluble fraction.

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Thymine starvation and enzyme synthesis

Cultures of *E. coli* strain 15T– suffer progressive and roughly parallel losses of viability and capacity to form the inducible enzyme β-galactosidase after about one generation

Abbreviations: RNA, ribonucleic acid; TMG, methyl-β-O-thiogalactopyranoside.