Inhibition of Nucleic Acid and Protein Synthesis in *Escherichia coli* by a New Triazenoimidazole.* (32503)

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Methyl 5(or 4)-(3,3-dimethyl-1-triazeno) imidazole-4(or 5)-carboxylate(1), designated NSC 87982 by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, has been reported to be a potent inhibitor of Gram-positive and Gram-negative bacteria, yeasts, filamentous fungi and algae *in vitro* and to protect mice against an experimental infection of *Staphylococcus aureus*(2). Microbiological assay of various mouse tissues indicates that not only are significantly high blood levels obtained following oral, intraperitoneal, or intravenous administration of the drug, but also that detectable concentrations of NSC 87982 are found in the brain and other organs of the mice(3). The broad-spectrum antimicrobial activity of NSC 87982 and the knowledge that it is being considered for clinical trial prompted a study of the mechanism of action of this compound.

Materials and methods. The inhibitory activity of NSC 87982 against numerous strains of *Escherichia coli* and *Streptococcus faecalis* which are resistant to various antimicrobial agents or radiation(4) was determined using previously described procedures (5); the spread-plate procedure(6) was used to determine the effect of metabolites (amino acids, B vitamins, purines, pyrimidines) on inhibition of *E. coli* by NSC 87982—details of this technique have been reported(7,8). Protein determinations were made by the method of Lowry *et al*(9). Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were determined by procedures previously described(10,11). By serial transfer in a simple glucose-salts medium containing increasing concentrations of NSC 87982, a culture of *E. coli* ATCC 9637 was obtained, (designated *E. coli*/NSC 87982) which is 30-fold resistant to the inhibitor.

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Results and discussion. Strains of *E. coli* or *S. faecalis* resistant to various antibiotics, antimetabolites, or irradiation were not cross-resistant to NSC 87982. Strains of *E. coli* ATCC 9637 or *E. coli* ATCC 11303 resistant to either 8-azaguanine or 6-thioguanine were observed to be approximately 10 times more sensitive to NSC 87982 than were the parent cultures from which they were derived. *E. coli*/NSC 87982 was completely resistant to 100 μg/ml of 8-azaguanine and partially resistant to 6-mercaptopurine ribonucleoside or porfiromycin. *E. coli*/NSC 87982 was >10-fold more sensitive to chloramphenicol and >100-fold more sensitive to glutamyl-γ-hydrazide than was the parent strain of *E. coli* from which it was obtained. Both the parent and NSC 87982-resistant cultures were equally sensitive to clinically useful antibiotics such as penicillin G, the tetracyclines, streptomycin, kanamycin, or neomycin. Equal sensitivity by the two cultures was observed to sparsomycin, azaserine, actinobolin, puromycin, and various antimetabolites such as 5-fluorouracil, 5-fluorodeoxyuridine, 6-mercaptopurine, 6-chloropurine, 2,6-diaminopurine, and the biological alkylating agents nitrogen mustard and 1,3-bis(2-chloroethyl)-1-nitrosourea.

Complete reversal of inhibition of 10 times the minimal inhibitory concentration of NSC 87982 for *E. coli* ATCC 9637 in liquid medium was observed with cysteine or homocysteine (each tested at a concentration of 100 μg/ml-concentrations, which were in themselves neither toxic nor stimulatory to the growth of *E. coli*). The effect was dose related and suggestive of a competitive reversal. Similar results were obtained in studies with *Saccharomyces cerevisiae* ATCC 8043, except that ascorbic acid or riboflavin were as effective as cysteine in reversing NSC 87982 inhibition of this yeast.

It was found that NSC 87982 inhibited both protein and RNA synthesis in *E. coli* ATCC 9637 (Fig. 1). Concentrations as low

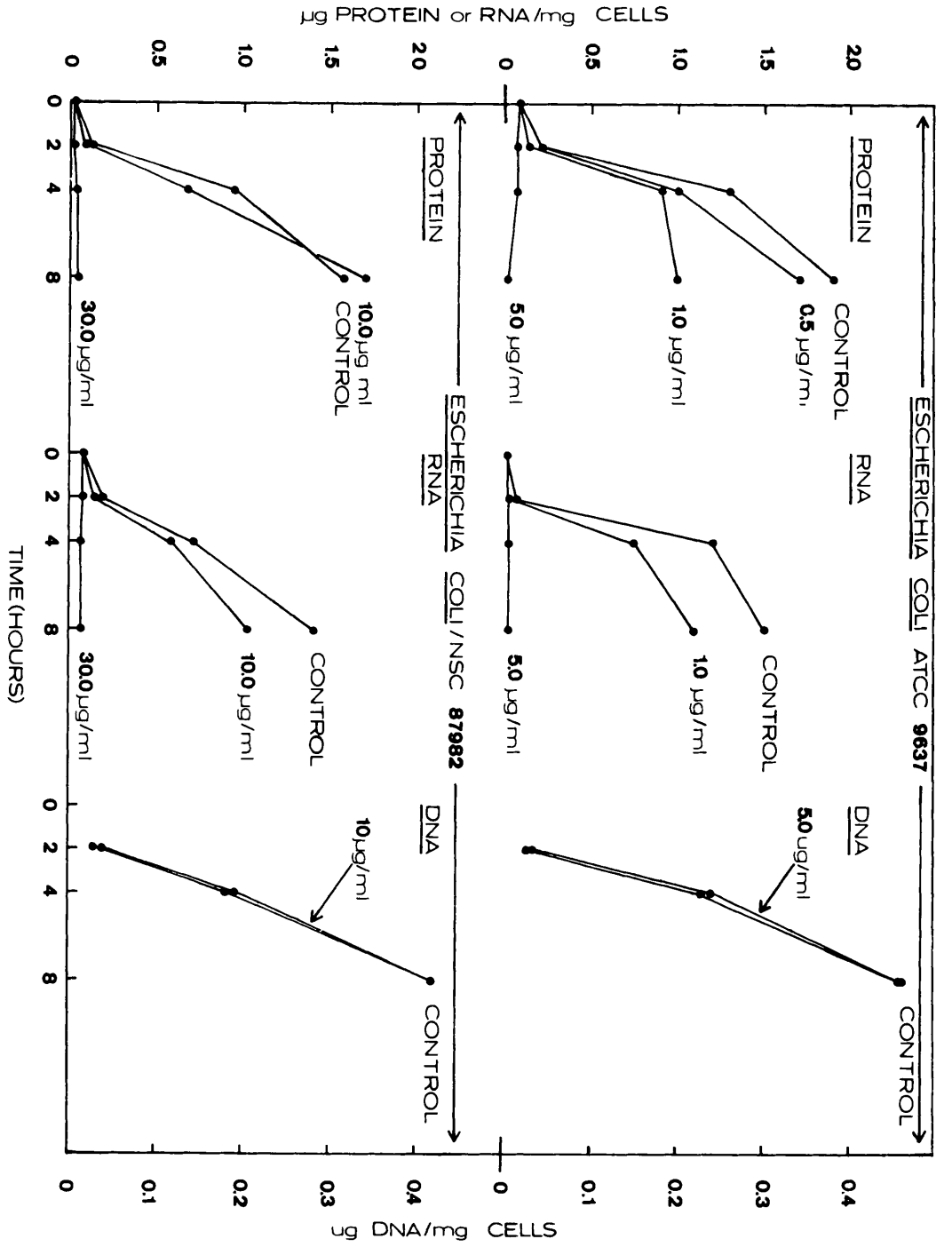

FIG. 1. Effect of NSC 87982 on DNA, RNA, and protein synthesis in *Escherichia coli* ATCC 9637 and *E. coli*/NSC 87982.

as 1.0 μg/ml were sufficient to markedly inhibit these synthetic processes, and a concentration of 5.0 μg/ml totally inhibited them. DNA synthesis in the same bacterium was unaffected. Synthesis of RNA and proteins in *E. coli*/NSC 87982 was unaffected by 5.0 μg NSC 87982/ml; however, higher concentrations (30 μg/ml) inhibited both of these processes. DNA synthesis was unaffected in this culture.

The precise mechanism by which NSC 87982 inhibits microorganisms is unknown. Based on the lack of cross resistance to other inhibitors of protein and/or RNA synthesis in *E. coli* such as actinobolin(12), puromycin(13), or sparsomycin(5), NSC 87982 may well be inhibitory at a different metabolic site.

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Vascular Leakage Induced by Horseradish Peroxidase in the Rat.* (32504)

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Horseradish peroxidase (HRP), a plant protein of approximately 40,000 molecular weight, has been used extensively during the past decade as a protein tracer in histological studies. Recently a relatively sensitive, specific, and simple method for localization of peroxidase with the electron microscope has been introduced(1) and used in a variety of tissues and experimental animals(1-5).

In the course of experiments on the localization of HRP in the mesothelium of the rat(3), it was observed that increased vascular permeability in venules occurred regularly after local or parenteral injection of the protein. Since one of the main ultrastructural applications of HRP is its use as a vascular tracer, further investigation of this phenomenon was undertaken and this communication presents the results of such studies.

Materials and methods. Adult male Sprague-Dawley rats, white male Hartley guinea pigs, and Swiss mice of both sexes were used. Increased vascular permeability was estimated by the local exudation of intravenously injected Trypan or Evans Blue in intradermal test sites. Rats were given 0.4 ml/100 g of a 1% solution of Trypan Blue into the tail vein. Guinea pigs and mice received 2.5% Evans Blue in a dose of 30 mg/kg. The test substances were injected intradermally, into the clipped abdominal skin, in a volume of 0.1 ml in rats and guinea pigs and 0.05 ml in mice, using a 26 gauge short bevel needle. Thirty minutes later the animals were sacrificed and the diameter of the blue staining on the undersurface of the skin was recorded.

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