Quinoxaline-1, 4-di-*N* Oxides
I. Inhibition of Deoxyribonucleic Acid Synthesis in *Escherichia coli* by 2,3-Dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide (34481)

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The potent *in vivo* activity of various quinoxaline-di-*N* oxides against diverse bacteria, *Entamoeba histolytica*, and the largest viruses of the psittacosis-lymphogranuloma group has been known for nearly two decades (1—5). Although originally prepared as potential antagonists of vitamin K activity, such antagonism in fact has not been demonstrated (3). Additional studies relevant to a possible mode of action for these interesting compounds apparently have been so limited that further reports cannot be found in the literature. We have recently studied the effect of 2, 3-dihydroxymethyl-quinoxaline-1, 4-di *N*-oxide (Fig. 1) on the incorporation of precursors of DNA, RNA, and protein synthesis in *Escherichia coli* 266 by radioisotopic techniques. The data indicate that the primary action of 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide is the inhibition of DNA synthesis.

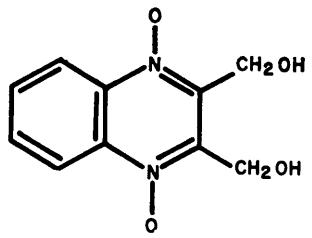

FIG. 1. 2,3-Dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide.

Materials and Methods. The *E. coli* strain 266 used in these studies has been maintained as a stock culture in this laboratory for several years and is routinely used in the *in vitro* and *in vivo* evaluation of experimental chemotherapeutics. For the present studies, the culture was grown in the synthetic medium of Witkin (6).

The following radioactive compounds were purchased from the New England Nuclear Corporation, Boston, Massachusetts: uniformly labeled thymidine-2-^{14}C (54.1 mCi/mmole); uracil-2-^{14}C (55.3 mCi/mmole); DL-leucine-1-^{14}C (30.2 mCi/mmole).

Incorporation of radioactive precursors. *E. coli* 266, from an overnight culture, was inoculated into synthetic medium in a shaker at 37° until the optical density (B and L 20; 600-mμ filter) reached 0.1. This culture was divided into three 40-ml portions, and thymidine-2-^{14}C, leucine-1-^{14}C, and uracil-2-^{14}C added to give final concentrations of 0.05 μCi/ml. The contents of each flask were divided into two 20-ml portions. One 20-ml portion received 2,3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide; the other portion served as a normal growth control. All flasks were incubated in a shaker water bath at 37° at 124 rpm. At 10-min intervals, 2-ml samples were removed and added to equal volumes of 10% TCA and held for 0.5 hr at 0°. The interval encompassed 1 hr. Cells were packed by centrifugation at 15,000 rpm for 20 min at 0°. The supernatant fraction was removed and discarded. The packed cells were resuspended in 2 ml of 1% TCA and were collected by Millipore (0.22 μ) filtration. The collected cells were washed four times in 1% TCA. After the final washing, the filter pad with its impinged bacteria was placed in a scintillation vial and allowed to dry overnight at room temperature. Fifteen milliliters of scintillation fluid [0.3% 2, 5-diphenyloxazole (PPO) and 0.01% *p*-bis-[2-(5-phenyloxazolyl)] -benzene (PoPoP)] was added to each vial and radioactivity was measured with a Tri-Carb liquid scintillation spectrometer (Packard Instrument Corporation, Chicago, Illinois). Data are presented as counts per minute.

TABLE I. *In Vitro* Activity of 2,3-Dihydroxymethyl-quinoxaline-1,4-di-*N*-oxide Against Representative Bacteria.

	Minimum inhibitory concentration (μg/ml)[a]
Escherichia coli	62
Shigella sonnei	62
Salmonella typhosa	132
Proteus vulgaris	62
Pseudomonas aeruginosa	122
Aerobacter aerogenes	62
Pasteurella multocida	266
Staphylococcus aureus	100
Streptococcus pyogenes	200

[a] Two-fold serial dilution technique in brain–heart infusion medium.

Results. Characteristic *in vitro* and *in vivo* activity of 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide against representative bacteria are presented in Tables I and II. It is obvious that 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide has greater activity *in vitro* and *in vivo* against gram-negative than gram-positive bacteria.

The studies utilizing isotopes were based on the assumption that the incorporation (into acid-insoluble materials) of thymidine and uracil represents DNA and RNA synthesis, respectively, and that leucine incorporation is a measure of protein synthesis. As shown in Fig. 2, protein, RNA, and DNA synthesis proceeded rapidly in the control. After 60 min of incubation, the amount of radioactivity incorporated into acid-insoluble material had increased approximately 10-fold for the synthesis of protein, 6-fold for the synthesis of RNA, and 9-fold for the synthesis of DNA. In the 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide-treated cultures, there was little, if any, apparent effect on protein and RNA synthesis during the 60 min of incubation (Fig. 2). In contrast, the culture exposed to 2, 3-dihydroxymethyl-quinoxaline-1,4-di-*N*-oxide at 50 μg/ml, which is approximately the bactericidal concentration, showed a diminution in the uptake of thymidine-2-^{14}C within 10 min and this became very pronounced at all later time intervals (Fig. 3). At the end of 60 min, the count of thymidine-2-^{14}C uptake in the 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide-treated *E. coli* only amounted to about 60% that shown by the control. The presence of drug at 100 and 200 μg/ml produced marked inhibition upon thymidine-2-^{14}C incorporation into DNA, and this effect was evident within 10 min (Fig. 3). After 60 min, the count of thymidine-2-^{14}C, in the drug-treated culture amounted to about 16% of that demonstrated in the control cultures. In effect, it would appear that this amount of thymidine-2-^{14}C was taken up within 20 min of incubation and this remained approximately constant over the 60-min period.

TABLE II. *In Vivo* Activity of 2,3-Dihydroxymethyl-quinoxaline-1,4-di-*N*-oxide Against Representative Bacterial Infections in Mice.

Infection	Protective dose 50% (mg/kg)[a]	
	Oral	Subcutaneous
Escherichia coli	12.5	11
Pasteurella multocida	18	16
Streptococcus pyogenes	180	200

[a] Intraperitoneal infection. Dosing at ½, 4, and 24 hr post infection.

Discussion. The *in vivo* activity of 2, 3-dihydroxymethyl-quinoxaline-1,4-di-*N*-oxide encompasses members of the true bacteria, protozoa, and viruses of the psittacosis-lymphogranuloma group. One might anticipate, therefore, that the target of such activity would be a process (or processes) common to diverse cell types and of prime importance in basic cell function. That the current studies have placed the site of activity of this drug in the inhibition of DNA synthesis is entirely consistent with this view.

The data clearly indicate that 2, 3-dihydroxymethyl-1,4-quinoxaline-di-*N*-oxide has little effect upon protein synthesis and the synthesis of RNA. The specificity of drug inhibition is upon the synthesis of DNA although this might be a direct or indirect effect. It is evident that such inhibition is not merely one manifestation of general metabolic failure.

Summary. The effect of 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide on the in-

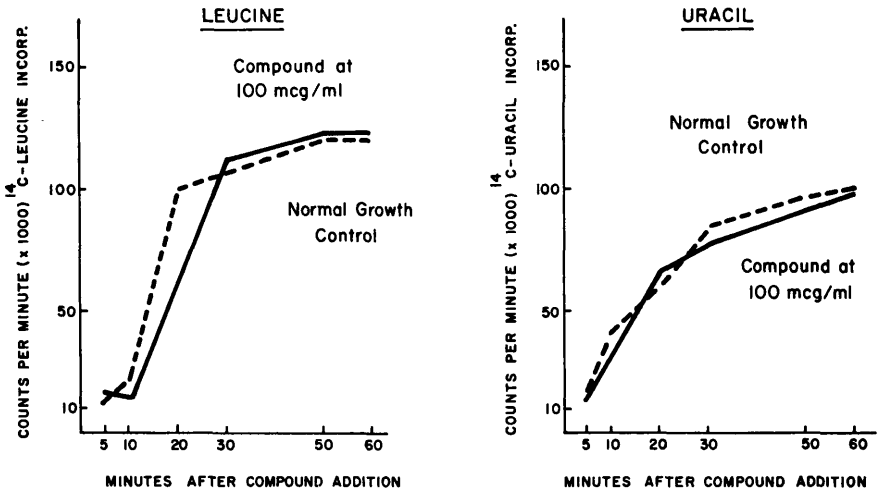

FIG. 2. Effect of 2, 3-dihydroxymethyl-quinoxaline-1, 4-di-*N*-oxide at 100 μg/ml on protein synthesis (^{14}C-leucine incorporation) and RNA synthesis (^{14}C-uracil incorporation) in *Escherichia coli* 266.

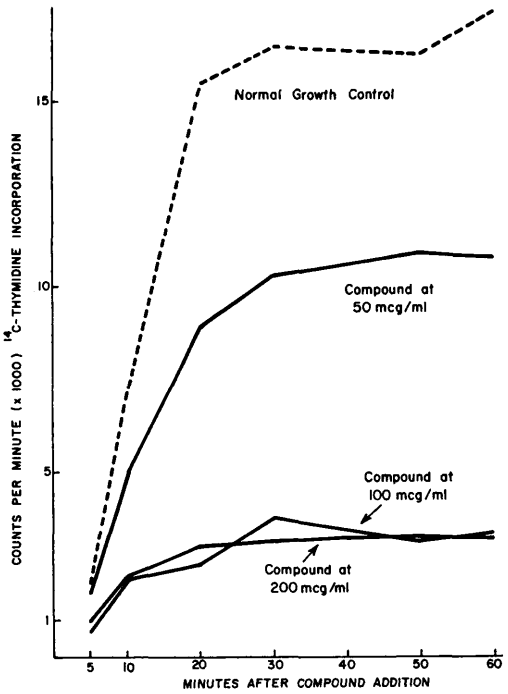

FIG. 3. Effect of various concentrations of 2, 3-dihydroxymethyl-quinoxaline-1,4-di-*N*-oxide on DNA synthesis (^{14}C-thymidine incorporation) in *Escherichia coli* 266.

corporation of precursors of DNA, RNA, and protein synthesis in *Escherichia coli* 266 was studied using radioisotopic techniques. The data clearly indicate that the activity of 2, 3-dihyroxymethyl-quinoxaline-1, 4-di-*N*-oxide is in the inhibition of DNA synthesis although whether the effect is direct or indirect has not been clarified yet.

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