The Inhibition of Organic Nitro-Reductase by Aureomycin in Cell-Free Extracts. (20244)

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Several reports have recently appeared on the effect of aureomycin on various enzymatic reactions in mammalian tissue. Loomis(1) observed that the antibiotic specifically depressed phosphorylation in normal mitochondria without, however, inhibiting respiration. Brady and Bain(2) reported that aureomycin similarly uncoupled oxidative phosphorylation in rat liver and brain. On the other hand, Van Meter and Oleson(3) found that aureomycin, in the absence of citrate, completely inhibited the respiration of whole rat liver homogenates within 10 minutes. In the presence of citrate, the rate of oxygen consumption did not start to decline until after 30 minutes, and an additional 30 minutes elapsed before the inhibition approached that of the citrate-free preparations.

In contrast to these effects of aureomycin on mammalian tissue, the antibiotic has hitherto not been found to inhibit cell-free enzyme systems derived from bacteria. Such an effect is, however, described in the present paper. Smith and Worrel(4) found that whole cell preparations of *E. coli* can reduce the nitro group of chloramphenicol to the corresponding arylamine. Egami, Ebata, and Sato(5) extracted a soluble enzyme from *Streptococcus hemolyticus* which mediated this reduction. In this laboratory cell-free extracts of *E. coli* were found to have a similar effect; and in both whole cell preparations and extracts, aureomycin inhibited the reduction to arylamine of the nitro groups of chloramphenicol and p-nitrobenzoic acid.

Methods. *E. coli* (E-26) was grown in a peptonized milk medium. Fifteen grams of Bacto-peptonized milk were autoclaved for 30 minutes in 900 ml of distilled water. 7.5 g of K_2HPO_4 were dissolved in 100 ml of tap water and the pH was brought to 7.2-7.4 by the addition of H_2SO_4. After autoclaving, the phosphate solution was added aseptically to the autoclaved peptonized milk solution and 0.1 ml of sterile tributyl citrate was added to the mixture. Twenty liter amounts of culture were grown overnight with continuous aeration at 37°C. The cells were harvested by Sharples centrifugation and washed once with cold distilled water. The wet paste of washed cells was stored at —10°C with no loss of activity for periods up to 4 weeks. Cell-free extracts were prepared by grinding 4 g wet weight of the washed cells with 8 g of Alumina A-301 (6). The paste was extracted with 20 ml of cold water and centrifuged in the cold at 27,000 x g for 30 minutes. This extract was stable for at least 4 weeks if stored at —10°. The complete system, containing (a) either 0.003 M chloramphenicol or p-nitrobenzoate, (b) 0.05 M phosphate or tris buffer at pH 7.0-7.5, (c) 0.005 M cysteine, (d) 10-100 μg aureomycin HCl/ml, and (e) 1.5 ml of cell-free extract in a total volume of 5 ml was incubated at 37°C for one hour. Arylamine was then determined by a modification of the Bratton-Marshall technic(4).

Results and discussion. Table I, Exp. 1, shows the effect of various concentrations of aureomycin-HCl on the reduction of 1 mg/ml chloramphenicol under aerobic conditions. Similar results were obtained when the reaction was run anaerobically. Even at concentrations of aureomycin as low as 10 μg/ml, the inhibition of the reduction was significant. In other experiments, the inhibition by 50 μg/ml was as large as 85%. Essentially similar results were obtained using p-nitrobenzoic acid as the reducible substrate.

Cysteine proved to be necessary for the reduction of the nitro compounds by the *E. coli* extract and no significant amount of diazotizable amine accumulated in its absence. Equivalent concentrations of pyruvate and

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TABLE I. Inhibition of Chloramphenicol Reductase by Aureomycin.*

Exp. No.	Aureomycin-HCl, μg/ml	Chloramphenicol, μg/ml	Optical density†	% inhibition
I	0	0	.015	—
	0	1000	.660	—
	10	"	.380	43
	20	"	.320	51
	30	"	.272	59
	50	"	.279	58
	100	"	.244	63
II	0	100	.437	—
	100	"	.177	60

* Experimental details cited in text.

† At 550 mμ in the Coleman Junior Spectrophotometer. At this wavelength, 4 μg of p-aminobenzoic acid had an optical density of 0.160.

succinate were 1/3 as active and glutathione was approximately 1/5 as active as cysteine; while malate, fumarate, and α-ketoglutarate were inactive. Aureomycin inhibited the reduction to the same extent in all 4 cases. In the absence of the extract, cysteine did not itself reduce the nitro compounds even at a final concentration of 0.05 M, 10 times that used in the present experiments. It may be noted that although the *E. coli* extract was found in manometric studies to catalyze the oxidation of cysteine, aureomycin in concentrations which inhibited reductase activity, had no effect on that cysteine oxidation.

In Exp. 1, Table I, the amount of arylamine formed represents approximately 2% of the total chloramphenicol (1000 μg/ml). In similar experiments with chloramphenicol at 100 μg/ml (Exp. 2, Table I), 10 μg of arylamine were formed in one hour and 18 μg in 2 hours, and the degree of inhibition by aureomycin was essentially the same as when 1000 μg/ml chloramphenicol was used.

Under the same conditions as those used in the experiments of Table I, terramycin had little effect on the nitro-reductase activity of *E. coli* extracts. This result is surprising in view of its recently reported isomorphism with aureomycin(7).

In view of the reports that aureomycin uncouples oxidative phosphorylation in mammalian tissue(1,2), the effect of 2,4-dinitrophenol on the reduction of the nitro groups of chloramphenicol and p-nitrobenzoic acid by the cell-free bacterial extract was investigated. The reduction of these compounds was inhibited to the same extent by dinitrophenol as by aureomycin. Studies are in progress on the possible similarity in the underlying mechanism of these two inhibitors, and on the role of phosphate in the system.

Summary. 1. Aureomycin-HCl in concentrations of 10-100 μg/ml inhibits the reduction to arylamine of the nitro groups of chloramphenicol and p-nitrobenzoic acid by cell-free extracts of *E. coli* (E-26). 2. Under similar conditions, terramycin has little effect on the reduction. 3. 2,4-dinitrophenol inhibits the reduction of the organic nitro compounds to the same extent as aureomycin. 4. Studies are in progress on the possible similarity in the underlying mechanism of inhibition by aureomycin and dinitrophenol.

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