Inhibition of Adaptive Enzyme Formation by Antimicrobial Agents. (18546)

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Despite the well-known capacity of chemotherapeutic and antibiotic agents to inhibit the growth of selected micro-organisms, the fundamental mechanisms involved in this process are poorly understood(1). The present studies indicate that the formation of certain adaptive enzymes is inhibited by several antimicrobial substances which interfere with multiplication of *Escherichia coli*. This effect provides a type of indicator reaction for studying the action of such substances on certain basic cellular processes.

Materials and methods. Suspensions of *Escherichia coli* (a freshly isolated strain furnished by the Department of Bacteriology, Army Medical Service Graduate School) were prepared from continuously aerated cultures grown for 10 hours at 37°C in brain heart infusion broth (Difco) containing 0.2% glucose. When cells adapted to lactose, maltose, arabinose, or acetate were required, they were prepared from cultures grown in a similar broth medium in which lactose, maltose, arabinose, or acetate had been substituted for glucose. Cells obtained in this manner were washed 3 times in physiological saline solution and were then aerated by a bubbling stream of air for 1 hour at room temperature prior to use. Oxygen consumption was measured in the conventional Barcroft-Warburg respirometer (32°C, 118 cycles per minute, 3 cm stroke, 3.0 ml final volume, and air as gaseous phase). Substrate concentration was uniformly 0.0017 M. Crystalline synthetic chloramphenicol and its L (+) optical isomer were obtained from Parke, Davis and Co.; terramycin hydrochloride was furnished by Chas. Pfizer and Co.; aureomycin hydrochloride was obtained in the form of the commercial intravenous preparation. The crystalline potassium penicillin G was a commercial product assaying 1435 units per mg; in the present work its concentration was expressed as mcg/ml of this commercial product.

Results. When lactose, maltose, arabinose, or acetate was added to a washed suspension of cells grown in the presence of glucose, oxygen consumption was at first equal to that of the endogenous control without substrate; however, after 40 to 120 minutes, oxygen consumption began, slowly at first but increasing exponentially until a final constant rate was attained. The results when plotted produced a curve typical of adaptive enzyme formation(2). There was no increase in number of viable organisms during the course of these experiments as determined by serial dilution plate count technic.

In contrast, when chloramphenicol (50 mcg/ml) was introduced into such a suspension prior to the addition of the substrate, the anticipated increase in oxygen consumption was not observed. Indeed, the oxygen consumption was maintained under these conditions at the level of the corresponding endogenous control (Fig. 1-B). A similar inhibi-

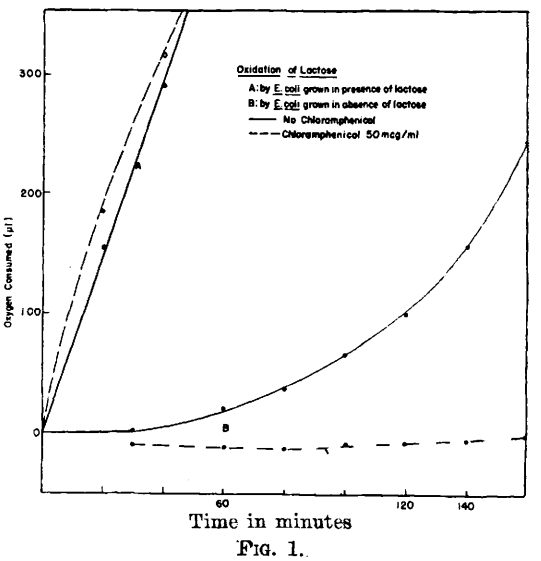

FIG. 1.

Inhibition by chloramphenicol of adaptation in *E. coli* for the oxidation of lactose and the failure of chloramphenicol to influence lactose oxidation in previously adapted organisms.

1. Pratt, R., and Dufrenoy, J., Antibiotics, Lippincott, 1949, p. 207.

2. Monod, J., *Growth*, 1947, v11, 223.

tory effect was elicited by chloramphenicol in tests on cells adapting to all 4 substrates; the data obtained from cell suspensions in the presence of lactose, illustrated in Fig. 1-B, are representative. Aureomycin (20 mcg/ml), terramycin (20 mcg/ml), and nitroacridine 3582 (50 mcg/ml) were as active as chloramphenicol in tests of this type, whereas penicillin (700 mcg/ml) failed to influence the phenomenon.

The distinction between inhibition of adaptive enzyme formation and interference with the action of the enzymes involved in the oxidation of the substrate was elucidated by the results of 2 types of experiment. In the first, cells were previously adapted by growth in a broth medium containing lactose, maltose, arabinose, or acetate instead of glucose. When 1 of these substrates was added to such a suspension of cells which had been grown in its presence, there was immediate and rapid oxygen consumption. Chloramphenicol (50 mcg/ml) did not inhibit this oxidation (Fig. 1-A).

In a second type of experiment, chloramphenicol was added at intervals to different vessels in a series during the adaptation of glucose-grown cells to lactose. Upon addition of chloramphenicol, the exponential increase in rate of oxidation was rapidly arrested. Oxygen consumption became a linear function with respect to time and the slope of the plotted curves was about proportional to the amount of adaptive enzyme present at the time chloramphenicol was added (Fig. 2). The results of this experiment confirm and supplement those of the preceding ones by demonstrating that chloramphenicol inhibits the formation of additional adaptive enzyme even though the adaptive process had already begun prior to its addition and that it does not inhibit the action of the adaptive enzyme already formed.

The L (+) optical isomer of chloramphenicol, a compound devoid of antibiotic activity (3), did not exhibit any of the effects just described.

The minimum amount of chloramphenicol

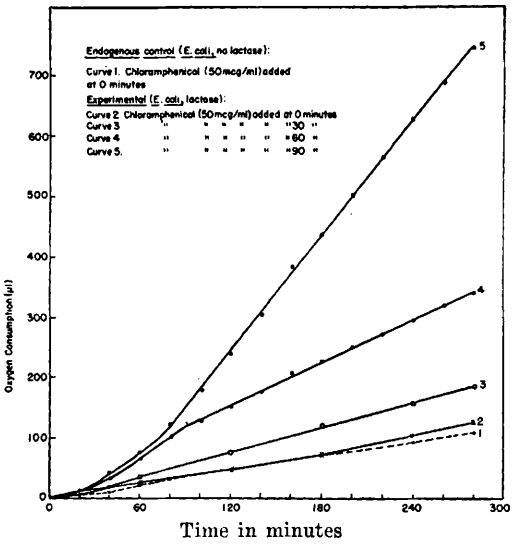

FIG. 2.

Arrest of adaptive enzyme formation in *E. coli* by chloramphenicol added at intervals during adaptation to lactose.

which would inhibit formation of adaptive enzymes in our strain of *E. coli* was approximately 5 mcg/ml. An escape from inhibition at this low drug concentration was noted after incubation of the mixture for about 2 hours in the Warburg apparatus. This escape was shown to be correlated with the reduction of the major portion of the added chloramphenicol to a biologically inactive amine, a reaction which has previously been observed in heavy suspensions of *E. coli*(4).

Washing chloramphenicol treated *E. coli* rendered them again capable of forming adaptive enzymes. *E.* coli which had been inhibited by soaking in 50 mcg/ml of chloramphenicol for 2.5 hours, in an experiment similar to that summarized in Fig. 1-B, responded in the normal manner as regards production of adaptive enzyme when washed and resuspended in lactose solution.

Discussion. The capacity of chloramphenicol to interfere with the formation of adaptive enzymes in *E. coli* appears to be related to the bacteriostatic properties of the antibiotic.* Thus, the minimal concentrations required to elicit the 2 phenomena are similar and the

3. Controulis, J., Rebstock, M. C., and Crooks, H. M., Jr., *J. Am. Chem. Soc.*, 1949, v71, 2463.

4. Smith, G. N., and Worrel, C. S., *Arch. Biochem.*, 1949, v24, 216.

L (+) optical isomer of chloramphenicol fails to produce either reaction. The minimal concentrations inhibitory to adaptive enzyme formation were not determined for terramycin, aureomycin, or nitroacridine 3582; however, the concentrations used and found effective were above the minimum bacteriostatic levels. On the other hand, adaptive enzyme formation was not inhibited by penicillin in a concentration 50 times that necessary to inhibit growth. Therefore, bacteriostatic action and inhibition of adaptive enzyme formation are frequently associated but are not inseparable properties. It may well be that the mode of action of penicillin differs from that of the other substances under investigation.

The failure of micro-organisms under the influence of chloramphenicol and other substances to form the adaptive enzymes under investigation does not account for their inability to multiply since the growth of *E. coli* with or without preformed adaptive enzymes is equally well inhibited by chloramphenicol. Adaptive enzyme formation, however, may be considered a special manifestation of a more basic cellular process—namely, protein metabolism. Experimental evidence for this point of view is furnished by Spiegelman *et al.*(5) who have shown that the adaptive formation of galactozymase in yeast is associated with the production of the specific protein component of the enzyme. In addition, Monod (6) and Reiner(7) have shown that dinitrophenol and azide, substances repeatedly demonstrated to inhibit synthetic processes, also inhibit adaptive enzyme formation. Interpretation of the inhibition of adaptive enzyme formation as either a primary or secondary interference with protein synthesis suggests a general site of action of the drugs which could readily explain their growth-inhibitory properties. Additional support for such a broad point of view is found in (a) our unpublished observations that chloramphenicol interferes with nitrogen assimilation in *E. coli,* (b) the studies of Woolley(8) suggesting that phenylalanine interferes to a limited extent with the growth-inhibitory effect of chloramphenicol, (c) the synergism and antagonism between chloramphenicol and various amino acids reported by Mentzer *et al.*(9), and (d) the recent report of Gale and Paine(10) showing that both chloramphenicol and aureomycin prevent the formation of cellular proteins in *Staphylococcus aureus.*

Summary. Chloramphenicol, aureomycin, terramycin and nitroacridine 3582 inhibit the formation of certain adaptive enzymes in *E. coli.* This effect *per se* is not responsible for the bacteriostatic activity of these substances, but it probably points toward a general interference with protein metabolism of the organism.

* Bacteriostatic concentrations were determined by incubating at 37°C for 24 hours serial dilutions of the drugs in brain heart infusion broth inoculated with the strain of *E. coli* used in this study. The minimal concentration preventing visible growth under these conditions was arbitrarily designated the bacteriostatic concentration. These levels were as follows: (1) chloramphenicol, 5 mcg/ml; (2) aureomycin, 2.5 mcg/ml; (3) terramycin, 5 mcg/ml; (4) nitroacridine 3582, 40 mcg/ml, penicillin G, 14 mcg/ml; and sodium sulfadiazine, >1000 mcg/ml.

5. Spiegelman, S., Reiner, J. M., and Morgan, I., *Arch. Biochem.,* 1947, v13, 113.

6. Monod, J., *Ann. Inst. Pasteur,* 1944, v70, 381.

7. Reiner, J. M., *J. Gen. Physiol.,* 1947, v30, 367.

8. Woolley, D. W., *J. Biol. Chem.,* 1950, v185, 293.

9. Mentzer, C., Meunier, P., and Molho-Lacroix, L., *Compt. Rend. Soc. de Biol.,* 1950, v230, 241.

10. Gale, E. F., and Paine, T. F., *Biochem. J.,* 1950, v47, 26.

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