INHIBITION BY TETRACYCLINES OF POLYURIDYLIC ACID DIRECTED PHENYLALANINE INCORPORATION IN ESCHERICHIA COLI CELL-FREE SYSTEMS

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Although inhibition of protein synthesis has been suggested as a mode of action of the tetracycline antibiotics (e.g. Gale and Folkes, 1953; Nikolov and Ilkov, 1961; Hash, 1963), little is known of the precise mechanism of the inhibition. Rendi and Ochoa (1962) mentioned that oxytetracycline had an effect similar to that of chloramphenicol on the transfer of aminoacyl residues to ribosomes in an Escherichia coli system. Franklin (1963) has recently reported that chlortetracycline reduced the incorporation of leucine into the ribosomal protein of cell-free systems from rat liver and E. coli, and demonstrated that the effect was on the transfer of amino acid from aminoacyl-sRNA to ribosomes. Oxytetracycline and tetracycline appeared to act in a similar way.

The present report describes the inhibition by several antibiotics of the tetracycline group of polyuridylic acid directed phenylalanine incorporation in cell-free systems derived from E. coli.

ABBREVIATIONS:

TC = tetracycline, OTC = 5-hydroxytetracycline (oxytetracycline), CTC = 7-chlortetracycline, DMCT = 6-demethyl-7-chlortetracycline, DDA-OTC = 4-dedimethylamino-5-hydroxytetracycline.

Poly U = polyuridylic acid, ATP = adenosine-5'-triphosphate, GTP = guanosine-5'-triphosphate, PEP = phosphoenolpyruvate, PK = pyruvate kinase, sRNA = soluble RNA (transfer RNA), S_{30} and S_{100} = supernatant fractions after centrifugation at 30,000 x g and 105,000 x g respectively.

METHODS:

E. coli cell-free extracts were prepared according to the methods of Nirenberg and Mattaei (1961). Determination of radioactivity of samples was carried out using the filter paper disc method of Mans and Novelli (1960). Charging of sRNA with amino acid and subsequent transfer of amino acid was based on the procedures described by Nathans and Lipmann (1961). Protein was determined by the Folin procedure of Lowry, et al. (1951).

RESULTS:

Inhibition by tetracycline: The inhibition by several tetracyclines of polyuridylic acid directed phenylalanine incorporation is shown in Figure 1. Ten γ/ml resulted in marked inhibition, and even at levels as low as 1 γ/ml, significant lowering of the rate and extent of incorporation was observed. There is a general similarity in the degree of inhibition observed with TC, OTC, CTC, and DMCT at both levels. Upon closer inspection of the data, however, the CTC and DMCT appeared to be somewhat more inhibitory than were the TC and OTC, both in affecting the initial rates of incorporation and the total incorporation at the end of the experiment. These results were reminiscent of many reports in the literature (e.g. Bohonos et al., 1957 and 1961; Garrod and Waterworth, 1960; Kirby et al., 1961) that the chlorinated tetracyclines are 2-3 times as potent against several species of bacteria as are TC or OTC. In addition, DDA-OTC has been found in our laboratories to have about 10% of the activity of tetracycline, and this degree of activity is also reflected in the cell-free system, in which it was observed that 10 γ/ml of DDA-OTC was required to inhibit to the same extent as 1 γ/ml of TC. The reason for these differences remains obscure, but the close parallel between the relative

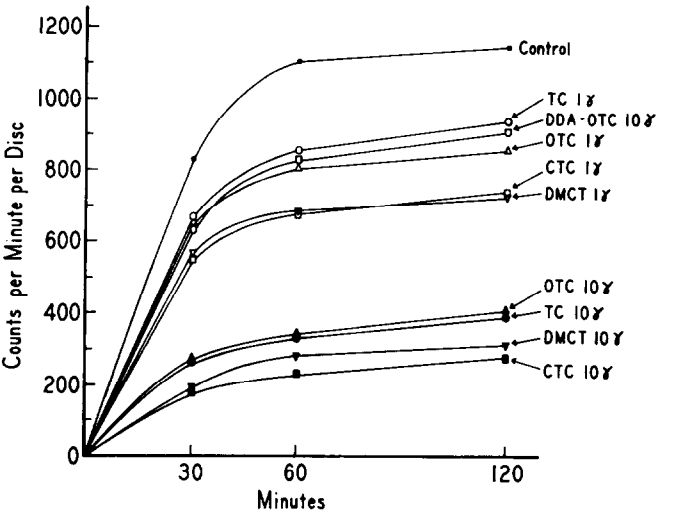

Figure 1. The effect of tetracyclines on polyuridylic acid directed phenylalanine incorporation in an E. coli cell-free system.

The complete reaction mixture (2.0 ml) contained: 0.05 μM phenylalanine (0.1 μc), 1 mg sRNA, 300 γ poly-U, 2 μM ATP, 0.5 μM GTP, 0.1 ml PK (100 γ protein), 10 μM PEP, 1 ml S_{30} fraction, in a total volume of 2.0 ml of "standard buffer" (Nirenberg and Matthaei, 1961) containing 0.01 M tris-HCl (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl and 0.006 M mercaptoethanol. Samples (0.1 ml) were taken at zero time and other appropriate intervals, applied to filter paper discs and treated according to the procedure of Mans and Novelli (1960). Each point in the figure represents the average of determinations for duplicate tubes.

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potencies of these antibiotics against whole cells and against the cell-free system would appear to lend support to the proposal that the primary mode of action of the tetracyclines is their effect on protein synthesis.

Localization of activity: Experiments were carried out in an attempt to localize the tetracycline effect in the protein biosynthetic pathway. In Table 1 are shown the results of an experiment demonstrating that tetracycline at levels of 10 or 100 γ/ml had no effect on the amount of labelled phenylalanine transferred to sRNA. The results plotted in Figure 2, however, indicate that the subsequent transfer of radioactivity

from the aminoacyl-sRNA to the ribosomes is markedly inhibited by tetracycline in an experiment using pre-charged sRNA. These results are analogous to those reported by Franklin for leucine incorporation into "endogenous" protein (1963).

Table 1.

γ/ml TC	Transfer of phenylalanine-C^{14} to sRNA (cpm/mg)
0	2510
10	2265
100	2450

The reaction mixtures were similar to that described in Figure 1, except that S_{100} was substituted for S_{30} and poly-U was omitted. After incubation, the RNA was extracted by the phenol method (Hoagland et al., 1958). Aliquots were pipetted onto filter paper discs and the radioactivity was determined by a liquid scintillation procedure similar to that described by Mans and Novelli (1960).

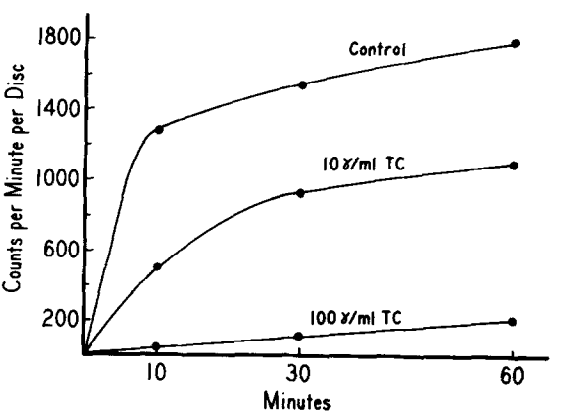

Figure 2. Transfer of C^{14}-phenylalanine from aminoacyl-sRNA to ribosomal protein.

For these experiments, C^{14}-phenylalanyl-sRNA with a high specific activity (ca. 25,000 cpm/mg) was prepared using high specific activity (140 mc/mM) C^{14}-phenylalanine.

Effect of tetracyclines on a cell-free system from a tetracycline-resistant E. coli: A strain of E. coli obtained from Dr. F. J. Sweeney, Jr. of the Jefferson Medical College was found to be resistant to concentrations of tetracycline greater than 100 γ/ml in a turbidometric assay. E. coli B, by contrast, was sensitive to ca. 0.2 γ/ml in the same test. Phenylalanine incorporation in a cell-free extract prepared from the resistant strain, however, was just as sensitive to the tetracyclines as were the preparations from E. coli B. In Figure 3 are compared the results with 1 and 3 γ/ml levels of OTC and CTC. Once again, the chlorinated tetracycline appears more inhibitory, requiring only 1 γ/ml to produce the same degree of inhibition as that resulting from 3 γ/ml of OTC. It would appear that the mechanism of resistance to the tetracyclines, at least in this particular strain, presumably resides at some point other than the protein synthesis pathway and perhaps is related to phenomena of permeability or adsorption.

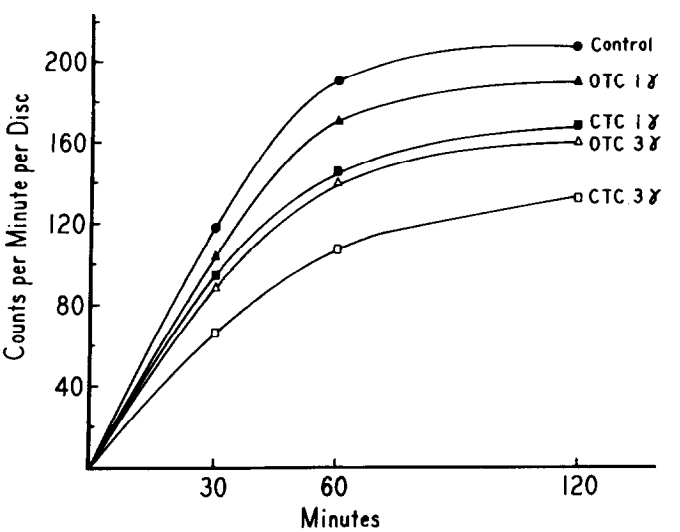

Figure 3. The effect of oxytetracycline and chlortetracycline on polyuridylic acid directed phenylalanine incorporation in a strain of E. coli resistant to the tetracyclines. (Details of the reaction are the same as those presented in the legend for Figure 1.)

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