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Action of the *N*-Trifluoroacetyl Analogue of D-Chloramphenicol

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The *N*-trifluoroacetyl analogue of chloramphenicol was found to inhibit the growth of *Escherichia coli* less than chloramphenicol, rather than more as previously reported by others. It also inhibits protein synthesis by *E. coli* and rat liver mitochondria less.

Chloramphenicol is a potent inhibitor of protein synthesis in whole-cell and cell-free systems of bacteria (1, 16) and in mitochondria (8, 11). It also inhibits deoxyribonucleic acid synthesis (23) and mitochondrial reduced nicotinamide adenine dinucleotide (NADH) oxidation (6) of mammalian cells and causes two types of bone marrow depression (18, 22). The reversible bone marrow depression has been ascribed to an inhibition of mitochondrial protein synthesis (8, 22). As it would be desirable to have an analogue of chloramphenicol that inhibits bacterial but not mitochondrial protein synthesis and that does not cause the hematological side effects, the action of many analogues on whole-cell and cell-free systems has been examined (3, 8, 10, 13, 17, 19). Generally, the inhibition of bacterial cell-free and mitochondrial protein-synthesizing systems are similar but cell permeability affects the action of some analogues against intact bacteria. Recently, it has been reported that the *N*-trifluoroacetyl analogue of chloramphenicol inhibited the growth of *Escherichia coli* more than chloramphenicol (10). We have, therefore, examined the effect of this analogue in *E. coli*, mammalian cells, and cell-free systems derived from them.

E. coli B/r was grown on Pennassay broth (Difco) and growth was followed by measuring light transmitted at 600 nm. Mouse L cells were grown as described previously (5).

Bacterial protein synthesis was measured by determining the incorporation of [^{3}H]leucine into hot acid-insoluble material using both *E. coli* and *E. coli* cell-free systems as described previously (8) with midlog phase cells. Protein synthesis was determined in whole cells by a 10-min incubation at 30°C in 50 μl of minimal growth medium containing 8×10^7 cells/ml, 0.2% glucose, and 0.85 mM L-[4,5-^{3}H]leucine (32 mCi/mmol). Protein synthesis by an S30 extract of *E. coli* was determined in incubations of 110 μl containing 8 μCi of L-[^{3}H]leucine (38 Ci/mmol)/ml and 0.3 mg of protein at 37°C for 20 min (8). Mitochondrial protein synthesis was measured by a modification (20) of earlier procedures (9). Incubations were at 30°C for 30 min with 17 μCi of L-[^{3}H]leucine (38 Ci/mmol) per ml. Mitochondria were also incubated with 0.1% digitonin (1.1 mg of digitonin/mg of mitochondrial protein) in a medium in which the adenosine 5'-diphosphate and succinate usually present were replaced by 2.3 mM adenosine 5'-triphosphate, 10 mM creatine phosphate, and approximately 50 μg of creatine kinase/ml.

NADH oxidase of four-times-washed rat liver mitochondria which had been frozen and thawed three times (7), the solubility of the trifluoroacetyl analogue in water and ethanol (7), radioactive counting (8), and protein determination (13) were performed as have been described previously. The source of materials has been described previously (5, 7, 8) except for the *N*-trifluoroacetyl analogue of chloramphenicol which was a gift of C. Hansch, Pomona College, Claremont, Calif.

The effect of chloramphenicol and its trifluoroacetyl analogue on the growth of *E. coli* and protein synthesis by whole bacteria, *E. coli* extracts, and rat liver mitochondria are shown in Fig. 1A–D, along with the level of antibiotic required for 50% inhibition. In all cases the trifluoroacetyl analogue was less inhibitory than chloramphenicol. The lesser inhibition in the *E. coli* cell-free system indicates that the results with whole cells cannot be explained by a lack of permeability to the analogue. Similarly, with mitochondria incubated in 0.1% digitonin (1.1 mg of digitonin/mg of mitochondrial protein), conditions in which the mitochondria should be disrupted (14), the trifluoroacetyl analogue was less inhibitory to protein synthesis as shown in Fig 1D. In contrast to the *N*-trifluoroacetyl analogue, the *p*-

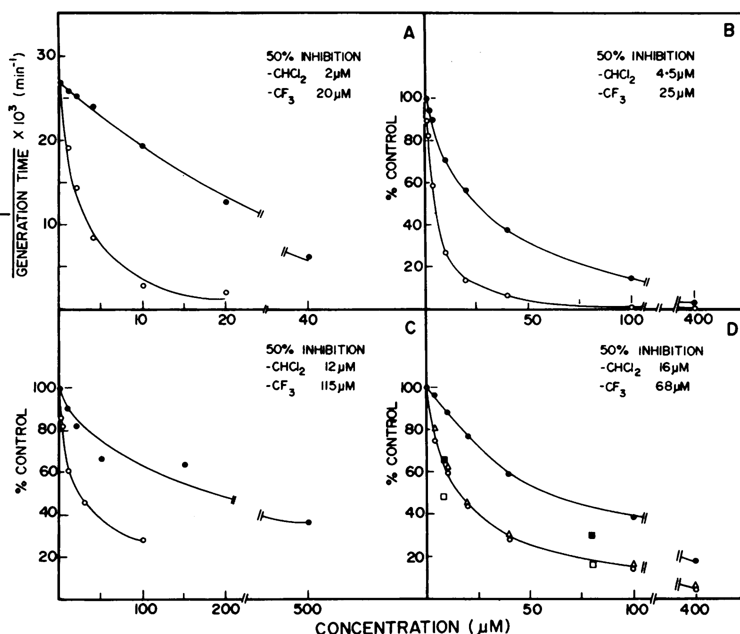

FIG. 1. *Effect of chloramphenicol and its trifluoroacetyl analogue on the growth of E. coli (A), protein synthesis by E. coli (B), E. coli extracts (C), and mitochondria (D). The controls were (A) a generation time of 37.2 min, (B) an incorporation of 1.8 × 10^4 cpm/10^8 cells per 10 min, (C) an incorporation of 1.8 × 10^4 cpm per mg of protein per 20 min, and (D) an incorporation of 6.0 × 10^4 cpm per mg of protein per 30 min. The values for 50% inhibition are given in each case. For E. coli cell-free protein synthesis the value given is for 60% inhibition, which is halfway between 100% and 20%, the approximate level of maximum inhibition. Symbols: ○, □, Chloramphenicol; ●, ■, trifluoroacetyl analogue; △, p-sulfamoyl analogue. The square symbols represent mitochondrial protein synthesis in the presence of 0.1% digitonin. The control value was 7.6 × 10^3 cpm per mg of mitochondrial protein which was 55% of the amount in the absence of digitonin.*

sulfamoyl analogue inhibited mitochondrial protein synthesis as much as chloramphenicol (Fig. 1D).

The trifluoroacetyl analogue inhibited NADH oxidation of frozen-thawed rat liver mitochondria 50% at 1.9 mM compared with 1 mM with chloramphenicol. The lesser inhibition by the trifluoroacetyl is a reflection of its lower relative solubility in ethanol (1.68 × 10^3 mmol/liter) compared with water (90 mmol/liter) than chloramphenicol (7) as would be expected from previous results (7). The effect of the trifluoroacetyl analogue and the sulfamoyl analogue of chloramphenicol on the growth of mouse L cells is shown in Fig. 2. The sulfamoyl analogue has the same effect on mitochondrial protein synthesis as chloramphenicol (8; Fig.1D), but inhibits NADH oxidation less (7). Although the trifluoroacetyl analogue inhibited growth more at first than the sulfamoyl analogue, the cells grew about the same number of generations before growth ceased.

This study was initiated because of a report that the trifluoroacetyl analogue of chloramphenicol was a stronger inhibitor of the growth of *E. coli* than chloramphenicol (10). This has not been confirmed. Garrett (personal communication) has rechecked his results and found that the earlier results arose from a misreading of the notation of the concentration of the analogue resulting in an apparent 10-fold greater inhibition. From the results of the present study and those of others (3, 4, 13, 17), it is suggested that any change in the dichloroacetyl side chain of chloramphenicol will decrease its inhibitory activity in both mitochondrial and bacterial cell-free protein-synthesizing systems. It has been suggested that chloramphenicol inhibits the peptidyl transferase reaction on bacterial ribosomes by inhibiting the binding of the aminoacyladenylyl terminus of charged transfer ribonucleic acid to ribosomes (3, 15). If this is correct, the stereochemical requirement for a dichloroacetyl side chain or its charge distribution may be critically important. In contrast, the *p*-nitro group can be replaced in some cases with no change in inhibitory strength (2, 8). The lesser inhibition of protein synthesis by mitochondria and *E. coli* extracts by the trifluoroacetyl analogue supports earlier

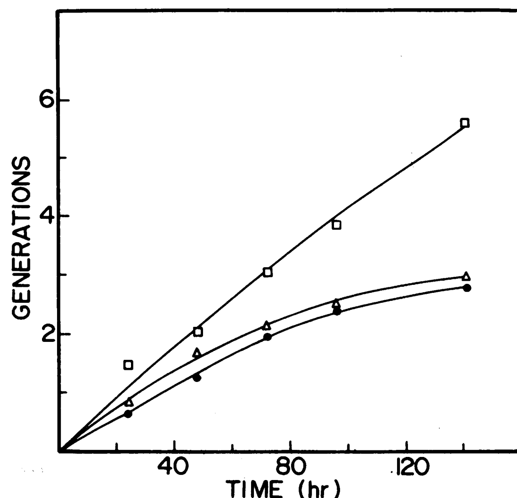

Fig. 2. *Effect of the sulfamoyl and the trifluoroacetyl analogue of chloramphenicol on the growth of L cells. The antibiotics were present at 0.28 mM. Symbols: □, Control; △, sulfamoyl analogue; ●, trifluoroacetyl analogue.*

studies with analogues that suggested a similar binding site for chloramphenicol on bacterial and mitochondrial ribosomes (8, 17).

We had previously found that the inhibition of growth of mammalian cells by chloramphenicol was due to the inhibition of mitochondrial protein synthesis and of NADH oxidation (5). The trifluoroacetyl analogue also inhibited the growth of mouse L cells as would be expected from its inhibition of mitochondrial protein synthesis. It is likely, then, that only an analogue that entered bacteria but not mammalian cells or mitochondria would have no hematological side effects.

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