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Streptococcal Tetracycline Resistance Mediated at the Level of Protein Synthesis

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The mechanism of tetracycline resistance was examined in strains containing each of the three previously identified resistance determinants in *Streptococcus* spp. Uptake of tetracycline was measured in tetracycline-sensitive cells as well as in cells containing each of the three resistance determinants. In cells containing *tetL*, uptake was not observed. However, in sensitive cells and cells containing either *tetM* or *tetN*, tetracycline was accumulated approximately 25-fold against a concentration gradient. Furthermore, there was no evidence for modification of intracellular tetracycline recovered from sensitive, *tetM*, or *tetN* cells. Protein synthesis in extracts derived from organisms containing *tetM* or *tetN* was resistant to tetracycline. In contrast, extracts of sensitive and *tetL* cells were sensitive to tetracycline.

Tetracyclines are broad-spectrum antibiotics with considerable activity against both gram-positive and gram-negative bacteria and exert a bacteriostatic effect by inhibiting protein synthesis. Widespread resistance to tetracycline has limited its clinical effectiveness against some groups of bacteria, especially streptococci. Unfortunately, the occurrence of tetracycline resistance is widespread among a number of Lancefield groups of streptococci of human and animal origin (2, 4, 15). We have previously identified three genetically distinct tetracycline resistance determinants in streptococci (3). These determinants, designated *tetL*, *tetM*, and *tetN*, were distinguished by their differential sensitivities to tetracycline antibiotics and DNA hybridization patterns. However, the basis for resistance due to these determinants has not been previously examined.

Previous analysis of naturally occurring tetracycline resistance in a number of other bacterial systems, e.g., *Escherichia coli* (12), *Staphylococcus aureus* (19), *Bacteroides fragilis* (7), and *Bacillus subtilis* (6), has indicated that drug resistance is mediated by decreased accumulation of the drug by resistant cells. The single reported exception to this generality has been in *Streptomyces rimosus* (18), the organism which produces tetracycline.

In this report, we characterize the basis of resistance in cells containing the streptococcal *tetL*, *tetM*, and *tetN* resistance determinants. This work demonstrated that, in contrast to sensitive cells, streptococci containing *tetL* do not accumulate tetracycline. However, cells containing *tetM* or *tetN* determinants transport tetracycline at the same rate and to the same extent as do sensitive cells. This idea is consistent with the demonstration that cell-free systems derived from *tetM* or *tetN* are resistant to tetracycline in vitro. Resistance in strains bearing the latter determinants apparently reflects elaboration of a factor rendering protein synthesis insensitive to intracellular drug.

(A preliminary report of this work has appeared [V. Burdett, Plasmid **13**:223, 1984].)

MATERIALS AND METHODS

Strains. For transport experiments, *Streptococcus faecalis* (*faecium*) ATCC 9790, which was obtained from the American Type Culture Collection, was used. A spontaneous mutant resistant to rifampin (ATCC 9790r) was isolated by plating a 10-fold concentrated broth culture on solid medium containing 100 μg of the antibiotic per ml. ATCC 9790r derivatives containing plasmids representative of *tetL* (pMV158), *tetM* (pAM211), and *tetN* (pMV120) were introduced by conjugation from JH203(pMV158 pIP501), JH203(pAM211), and JH203(pMV120) (3).

Media. *S. faecalis* 9790 and its tetracycline-resistant derivatives were routinely grown in KTY medium which contained 1% K_2HPO_4, 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% yeast extract (Difco), and 1% glucose (10). Medium KMTY is reported to have low phosphate content (11) and was used to investigate the effects of arsenate on tetracycline accumulation.

Determination of antibiotic resistance. The susceptibility of *S. faecalis* isolates to tetracycline and minocycline were determined with serial dilutions of antibiotic (22) in KTY broth. Growing cultures were diluted to approximately 10^5 CFU/ml and mixed with KTY broth containing various concentrations of antibiotic. The lowest concentration of antibiotic which completely inhibited visible growth after 24 h was taken as the MIC for each strain. In some experiments, optical densities were measured with a Klett-Summerson colorimeter.

Inducibility was tested by challenging cultures grown in the presence and absence of subinhibitory concentrations of tetracycline and monitoring the effect of drug challenge on subsequent growth. Cultures growing logarithmically in the absence (A) or presence (B) of a subinhibitory concentration (0.5 μg/ml) of antibiotic were split into two subcultures. Cultures A1 and A2 contained no antibiotic, and growth was monitored for 45 to 60 min, at which time culture A2 was challenged with tetracycline (2 μg/ml for *tetL* and 20 μg/ml for *tetM* and *tetN*). Cultures B1 and B2 were maintained at 0.5 μg of tetracycline per ml for 45 to 60 min, at which time culture B2 received the tetracycline challenge (as for culture A2). The unchallenged cultures received a mock challenge of water in place of tetracycline. Growth was determined by monitoring the increase in A_{590}.

Uptake of [^{3}H]tetracycline. Overnight cultures were diluted and allowed to grow to mid-log phase (A_{590} = 0.5 to 0.8). After washing the cells with 50 mM K–*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.4) at room temperature, the cells were suspended in 0.5 volume of KTY medium. After a 15-min preincubation at 37°C in the presence or absence of 1% glucose as indicated,

[^{3}H]tetracycline (0.6 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added to a final concentration of 5 μM. Samples (0.1 ml) were removed periodically and added to 3.0 ml cold saline (0°C) and filtered through Metricel GN-6 filters (Gelman Sciences, Inc., Ann Arbor, Mich.). After five washes with cold saline, the filters were added to 1 ml of 1 N HCl in a scintillation vial and heated to 65°C for 10 min and cooled, and ^{3}H was determined in 10 ml of Aquasol (New England Nuclear). Preliminary studies had shown that these filters gave the lowest background of [^{3}H]tetracycline binding, whereas backgrounds were much higher with cellulose acetate-cellulose nitrate or glass fiber filters. Transport is expressed as the intracellular concentration of tetracycline estimated from the specific activity of the drug used and the internal volume of the cells (1.5 μl per mg of dry weight; F. M. Harold, personal communication). Ionophores and uncouplers were added 2 min prior to the addition of tetracycline or during uptake at the time indicated in the figure legend.

Analysis of intracellular [^{3}H]tetracycline taken up in cells. Cells were grown to A_{590} = 1.0 in KTY medium, centrifuged, and washed at room temperature with 5 volumes of 50 mM K-HEPES (pH 7.6)–0.4 mM $MgSO_4$, suspended in the same buffer at A_{590} = 5.0, and preincubated in the presence of 1% glucose at 37°C. [^{3}H]tetracycline was added to a final concentration of 5.0 μM, and uptake was allowed to proceed for 30 min. Cells were then diluted by addition of 30 volumes of 0°C buffer and collected by centrifugation. After two additional washes with 0°C buffer, the final cell pellet was suspended in 0.5 volume of ice-cold (10 mM) ammonium bicarbonate. Tetracycline was extracted with ice-cold *n*-butanol, concentrated by lyophilization, and dissolved in a small volume of *n*-butanol. Approximately 80% of the labeled tetracycline was recovered from the cells in the ammonium bicarbonate and butanol fractions combined. The butanol fraction contained 45% of the tetracycline recovered from the cells; this proportion was also observed when radiolabeled tetracycline was added to ammonium bicarbonate and carried through the extraction procedure.

Samples were applied to Whatman, Inc. (Clifton, N.J.), no. 1 paper (impregnated with 0.1 M EDTA [pH 8.0] and dried) and subjected to descending chromatography with butanol-ammonium hydroxide-water (5:1:4) (21). Unlabeled, authentic tetracycline was included in each lane and was visualized by virtue of its fluorescence under UV illumination. Chromatograms were cut into strips, and ^{3}H was determined by liquid scintillation counting.

Assay for protein synthesis in vitro. The assay for protein synthesis in vitro was based on the method of Nirenberg (17). Cultures were grown in KTY broth, harvested during the logarithmic phase, washed with buffer A (0.05 M Tris [pH 7.6], 0.03 M NH_4Cl, 0.017 M $Mg(OAc)_2$, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride), and stored frozen at −70°C. Cells were disrupted by grinding with mortar and pestle for 15 min with 2 parts (wt/wt) of alumina powder. Disrupted cells were extracted with 2 volumes of buffer A, and alumina and large cell debris were removed by centrifugation for 20 min at 12,000 × *g*. The supernatant fraction (S30) recovered after centrifugation at 30,000 × *g* for 30 min (17) was aliquoted, quickly frozen, and stored at −20°C. Extracts contained less than 0.2 CFU per assay. Cell-free protein synthesis reactions (0.1 ml) were those of Traub et al. (20), except that synthesis was directed by endogenous mRNA, and labeled amino acids were [^{3}H]threonine, [^{3}H]phenylalanine, and [^{3}H]aspartate. Synthesis was terminated after 60 min by addition of 3 ml of 10% trichloracetic acid and heating at 90°C for 15 min. Acid precipitates were cooled and filtered onto Whatman GF/A filters, which were dried and counted. Assays were found to be linear with both extract concentration and time.

Reagents. Valinomycin and *N*,*N*′-dicyclohexylcarbodiimide were purchased from Sigma Chemical Company (St. Louis, Mo.); nigericin and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were from Calbiochem-Behring (La Jolla, Calif.). All the foregoing were added in ethanol such that the final concentration of ethanol never exceeded 0.1%. All antibiotics were purchased from Sigma. Aqueous stocks of tetracycline (10 mg/ml) and nonaqueous stocks of minocycline (5 mg/ml in methanol) were prepared fresh daily. Concentrations were determined spectrophotometrically (14). [^{3}H]threonine was obtained from New England Nuclear, and [^{3}H]phenylalanine and [^{3}H]aspartate were purchased from Research Products International.

RESULTS

Susceptibility to tetracyclines. Previous analyses of R-factor-mediated tetracycline resistance in *E. coli* (12), *S. aureus* (19), *B. fragilis* (7), and *B. subtilis* (6) have demonstrated reduced accumulation of tetracycline by resistant cells. In *E. coli*, this has been shown to be the result of an energy-dependent efflux system. Since the mechanism of tetracycline resistance has not been previously examined in streptococci, uptake of tetracycline was explored in *S. faecalis* ATCC 9790r and derivatives containing the three tetracycline resistance determinants (*tetL*, *tetM*, and *tetN*).

Because of their structural and metabolic simplicity, streptococci are well suited for studies involving possible transport phenomena. *S. faecalis* lacks internal energy reserves, does not carry out oxidative phosphorylation, and is sensitive to ionophores and other agents which de-energize the membrane (5). During ordinary growth, the cells lack cytochromes and rely entirely on glycolysis and substrate level phosphorylation for metabolic energy (9). *S. faecalis* (*faecium*) ATCC 9790 has been used extensively to study transport of metabolites and cations and thus provides a well-characterized background for studying tetracycline transport.

ATCC 9790r and derivatives containing *tetL*, *tetM*, and *tetN* plasmids exhibited patterns of resistance to tetracycline antibiotics similar to those of other streptococcal strains (3). In particular, ATCC 9790r containing pMV158 (*tetL*) had an MIC of 20 μg of tetracycline per ml, 40-fold more resistant than the sensitive host, and did not mediate resistance to minocycline, a finding consistent with previous results in other genetic backgrounds (3). Class M (pAM211) and N (pMV120) plasmids mediated high-level resistance to tetracycline up to 200 μg/ml and increased resistance to minocycline from 0.04 to 10 μg/ml. Pregrowth of any of the resistant strains in the presence of subinhibitory concentrations of tetracycline had no effect on the resistance levels observed. This is in contrast to *E. coli*, in which the inducible resistance determinants showed increased resistance when the cells were first exposed to a subinhibitory concentration of tetracycline (8).

Based on our initial analysis of the effects of tetracycline on growth rate, we reported that *tetL*, *tetM*, and *tetN* were constitutively expressed (2). However, experiments described below suggested that exposure of cells containing *tetM* or *tetN* to tetracycline increased the level of resistant protein synthesis. Consequently, we reinvestigated this question at the level of growth response (Fig. 1) and found that, by careful examination of the growth rates of cultures

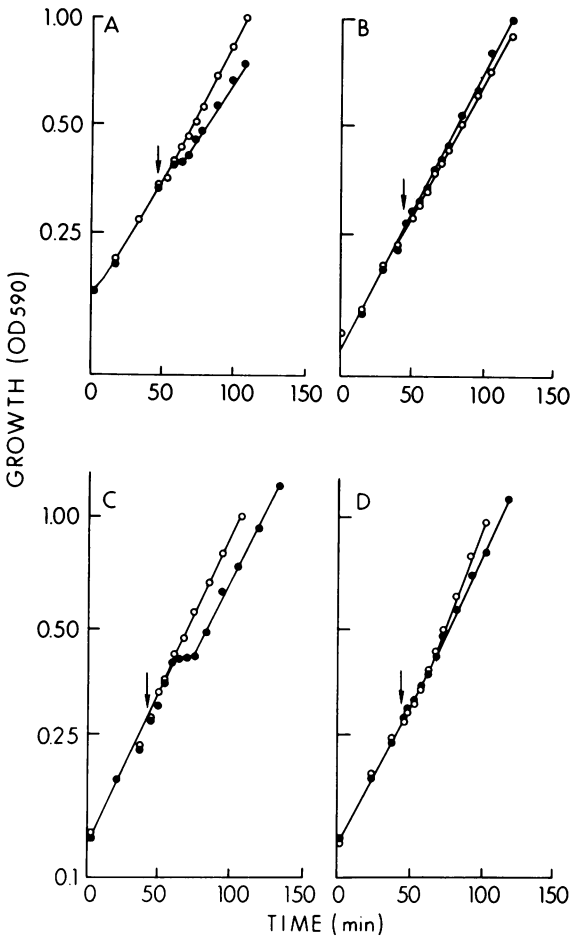

FIG. 1. Mode of expression of the tetracycline resistance genes *tetM* and *tetN*. Logarithmic-phase cells of ATCC 9790r containing pAM211 (A, B) and pMV120 (C, D) that were growing in the absence (A, C) or presence (B, D) of 0.5 μg of tetracycline per ml. For each strain, two cultures were grown, and tetracycline (20 μg/ml) was added to one flask (●) at the time indicated by the arrow but omitted from the second (○). Growth was recorded as an increase in the A_{590} of the culture.

challenged with tetracycline with or without prior exposure to subinhibitory concentrations of the drug, pMV158 (*tetL*) mediated constitutive resistance (data not shown), whereas pAM211 (*tetM*) and pMV120 (*tetN*) mediated inducible resistance. Induction of *tetM* and *tetN* was clearly observed in about half of the experiments, but higher levels of resistant protein synthesis were consistently observed when cells containing these determinants were grown in tetracycline (see below).

Transport of tetracycline by ATCC 9790r. Uptake of tetracycline in the sensitive strain (ATCC 9790r) exhibited properties of active transport (Fig. 2). The drug was accumulated against a concentration gradient, a phenomenon which was dependent on the presence of glucose as an energy source. Furthermore, as shown below, no modification of accumulated drug was detectable. In addition, uptake was reversible, as evidenced by exchange upon transfer of cells preloaded with labeled tetracycline to fresh medium with or without excess unlabeled tetracycline (Fig. 2d).

N,N'-dicyclohexylcarbodiimide, nigericin, CCCP, arsenate, and valinomycin can, under specific conditions, alter the ability of cells to carry out transport functions. In the presence of K^+, valinomycin was without effect on the rate or extent of uptake of tetracycline. However, under identical conditions, and as shown by others (1), we found that transport of threonine was blocked (data not shown). Since this ionophore collapses membrane electrical potential in the presence of potassium, these results indicate that, in contrast to threonine transport, membrane electrical potential appears to be unimportant for tetracycline transport. In contrast, *N,N'*-dicyclohexylcarbodiimide and arsenate, which interfere with the ability of streptococci to maintain a proton motive force across the cell membrane (9), inhibit active accumulation of tetracycline by sensitive cells. Agents which collapse the transmembrane pH gradient, such as CCCP (Fig. 2c) and nigericin (Fig. 2b), also blocked the accumulation of tetracycline, and CCCP induced rapid loss of tetracycline from cells which had previously been allowed to accumulate the drug (Fig. 2c). These results suggest that transport of tetracycline in streptococci is driven by the

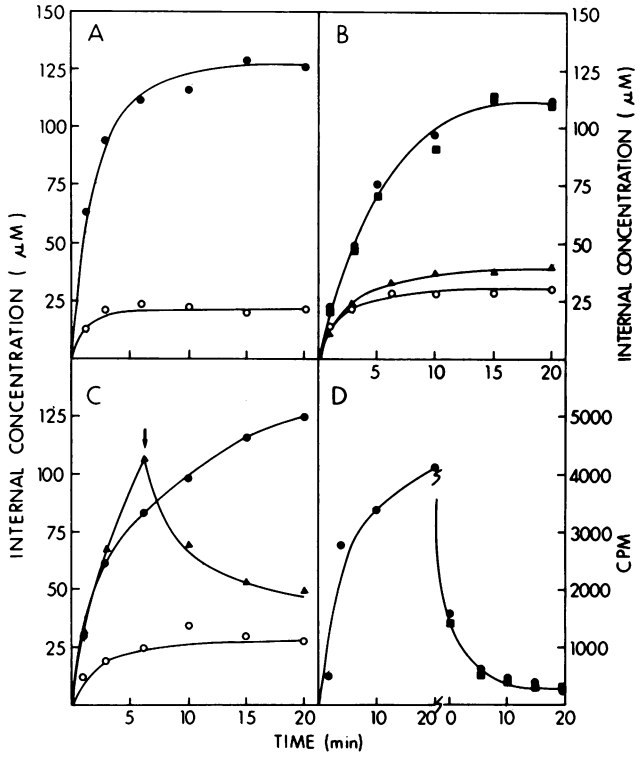

FIG. 2. Uptake of tetracycline by ATCC 9790r. Cells grown on KTY-glucose were assayed at A_{590} = 5.0 for their ability to accumulate drug in the presence of 5 μM external [^{3}H]tetracycline at 0 min. Samples were withdrawn at intervals into 3 ml of ice-cold saline, collected onto filters, and washed, and ^{3}H was determined by liquid scintillation counting as described in Materials and Methods. (A) Cells were incubated in KTY with (●) or without (○) glucose. (B) Cells were incubated in KTY-glucose (●) for 10 min, and 10^{-6} M valinomycin (■) 10^{-6} M nigericin (▲) was added 2 min before the addition of labeled tetracycline; the control was without glucose (○). (C) The cells were divided into three samples, which were treated as follows. The first (●) received [^{3}H]tetracycline. The second (▲) received labeled tetracycline at 0 min and 1.5×10^{-6} M CCCP at 6 min. A third sample was incubated without glucose (○). (D) Cells were incubated in KTY-glucose in the presence of [^{3}H]tetracycline. After 20 min, the cells were diluted in 0°C saline, washed, and suspended in fresh medium in the absence (●) or presence (■) of 50 μM unlabeled tetracycline. Cells at 0°C did not take up or exchange tetracycline. CPM, Counts per minute.

transmembrane pH gradient. These results are similar to our preliminary conclusions (V. Burdett, Plasmid 11:188, 1984) and those of Munske et al. (16), who measured tetracycline transport in streptococci by a fluorescence method.

Tetracycline accumulation in resistant cells. As previously reported, strains bearing pMV158 (*tetL*) remain sensitive to minocycline, whereas those containing pAM211 (*tetM*) and pMV120 (*tetN*) are minocycline resistant. These differences in susceptibility suggested differences in the resistance mechanism between *tetL* on the one hand and *tetM* and *tetN* on the other. When tetracycline accumulation was measured in ATCC 9790r cells containing *tetL*, *tetM*, or *tetN*, two patterns of accumulation were observed (Fig. 3). *tetL*-containing cells did not accumulate tetracycline over background levels observed in all cell types in the absence of glucose. However, tetracycline accumulation by cells containing *tetM* or *tetN* was comparable in both rate and extent to that observed in sensitive cells. As in sensitive cells, this accumulation was energy dependent, as shown by the requirement for added glucose.

Analysis of intracellular tetracycline. Energy-dependent uptake of tetracycline by resistant cells raised the possibility of drug modification within *tetM* and *tetN* cells to yield a derivative which was less active as an inhibitor of protein synthesis. However, the tetracycline accumulated by cells could be freely exchanged with tetracycline in the medium (Fig. 2d), and the activity of tetracycline recovered from sterile culture supernatants of resistant strains grown in the presence of tetracycline retained full biological activity (Fig. 4). In addition, accumulated tetracycline was extracted from within sensitive cells and *tetM* and *tetN* resistant cells and examined chromatographically (Fig. 5). As can be seen, radioactive tetracycline extracted from sensitive and resistant cells comigrated with species found in commercial samples of the unlabeled drug and radiolabeled tetracycline carried through the same extraction procedure. These findings indicate that resistance of cells harboring *tetM* and *tetN* is not mediated by drug modification.

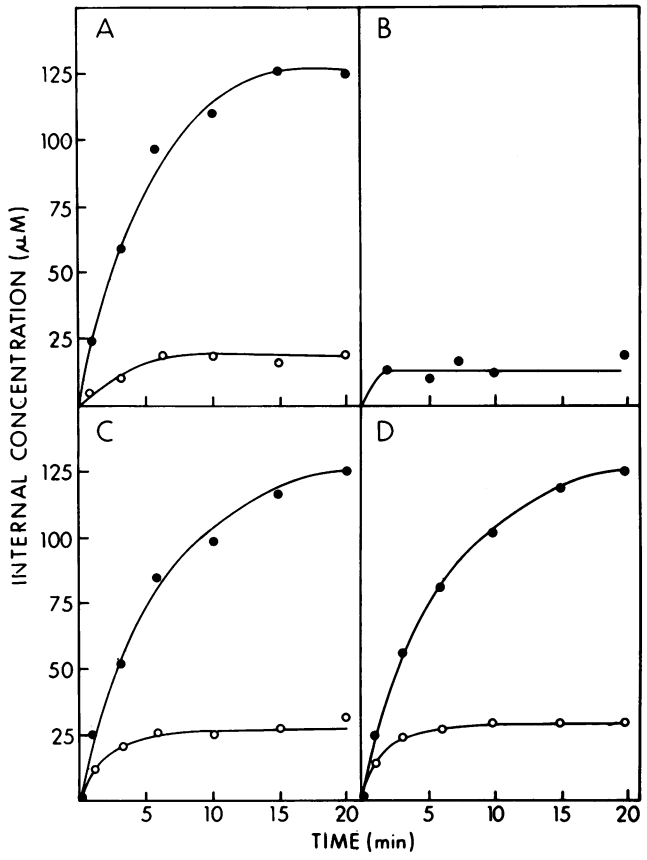

FIG. 3. Uptake of tetracycline by ATCC 9790r and resistant derivatives. Cells were tested for their ability to accumulate labeled tetracycline in the presence (●) or absence (○) of added glucose. (A) ATCC 9790r. (B) ATCC 9790r(pMV158) (*tetL*). (C) ATCC 9790r(pAM211) (*tetM*). (D) ATCC 9790r(pMV120) (*tetN*). Results with ATCC 9790r(pMV158) were the same in the presence or absence of glucose.

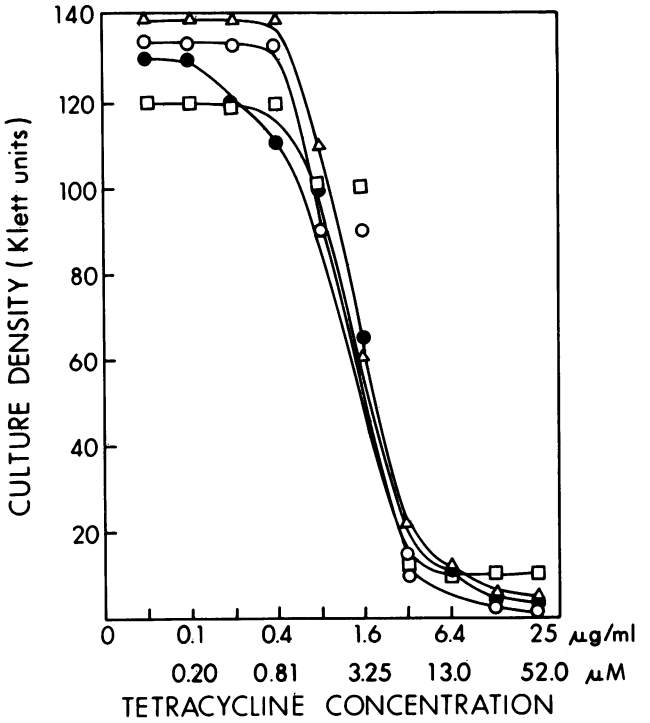

FIG. 4. Biological activity of tetracycline recovered from sterile culture supernatants of resistant strains. Cultures of strains containing *tetL* (□), *tetM* (△), or *tetN* (○) were grown in the presence of 25 μg of tetracycline per ml. Following overnight incubation, sterile culture supernatants were tested for the presence of antibiotic by twofold dilution test with ATCC 9790r as the indicator strain. The control (●) was sterile culture supernatant from JH2-2 (tetracycline-sensitive strain), to which fresh tetracycline was added to a final concentration of 25 μg/ml. Culture densities (Klett units) were measured after overnight incubation.

Tetracycline-resistant protein synthesis in vitro. The foregoing results suggested that tetracycline resistance in *tetM* and *tetN* cells might involve a mechanism in which protein synthesis is rendered insensitive to the presence of chemically authentic tetracycline. This possibility was addressed by analysis of protein synthesis in cell-free fractions prepared from drug-sensitive and -resistant cell types (Fig. 6). Protein synthesis in extracts prepared from sensitive ATCC 9790r or those containing the *tetL* uptake resistance system were very sensitive to inhibition by added tetracycline. In both cases, synthesis in extracts was inhibited 50% by tetracycline concentrations of about 10 μM. This can be compared with an intracellular concentration in excess of 100 μM achieved by transport of the drug into sensitive cells (Fig. 2). In contrast, 50% inhibition of in vitro synthesis in

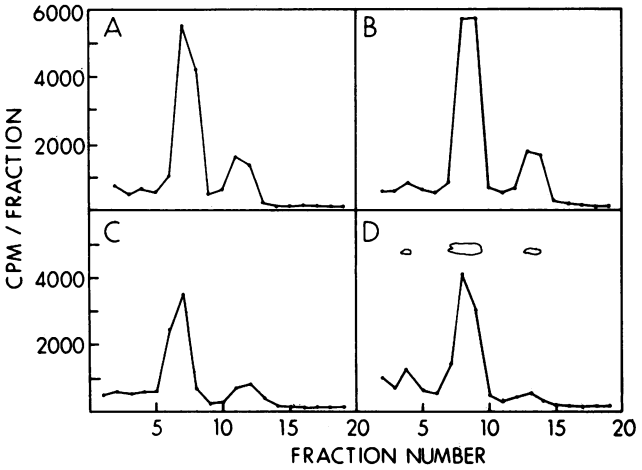

FIG. 5. Chromatography of intracellular [^{3}H]tetracycline recovered after accumulation. Cells were allowed to accumulate labeled tetracycline for 30 min in the presence of glucose. The cells were diluted in 0°C buffer and collected by centrifugation. Tetracycline was extracted from the cells with ice-cold *n*-butanol, concentrated by lyophilization, and analyzed by descending paper chromatography as described in Materials and Methods. Drug was extracted from (A) ATCC 9790r, (B) ATCC 9790r(pAM211) (*tetM*), (C) ATCC 9790r(pMV120) (*tetN*), and (D) a sample of buffer to which labeled tetracycline was added. Above the chromatogram in D are spots representing the position of unlabeled tetracycline and its breakdown products as visualized under UV light. Although not shown, the peaks in all chromatograms corresponded precisely with the unlabeled marker tetracycline.

tetM and *tetN* extracts was observed only at concentrations of about 300 μM.

This extent of resistance was observed in cells grown in the presence of tetracycline; when extracts were prepared from these cells grown in the absence of tetracycline, the midpoint of inhibition was reduced to about 30 μM. Although the extent of resistance was reduced about 10-fold relative to extracts from induced cells, these extracts consistently exhibited threefold higher resistance compared with extracts prepared from sensitive or *tetL* strains. These results clearly demonstrate that the protein-synthetic apparatus in cells harboring *tetM* or *tetN* determinants is resistant to inhibition by tetracycline. Furthermore, the tetracycline recovered after incubation with these extracts for 60 min was found to have retained full biological activity (data not shown).

DISCUSSION

Three tetracycline resistance determinants (*tetL*, *tetM*, and *tetN*) have been described in *Streptococcus* spp. (3). Although these determinants were originally distinguished genetically and by virtue of differential resistance to tetracycline and minocycline, it is shown here that *tetL* is also distinguished from *tetM*, *tetN*, or both at the level of mechanism of resistance. Indeed, this work describes a mechanism of R-factor-associated tetracycline resistance in a clinically significant pathogen that has the unusual property of being mediated at the level of protein synthesis.

It is known in other systems that energy-dependent transport of tetracycline is blocked in resistant strains, owing to the presence of a plasmid-mediated efflux system (6, 7, 12, 19). The streptococcal *tetL* determinant presumably mediates resistance by the familiar efflux system (unpublished data). Indeed, this appears to be the case; not only is accumulation of tetracycline blocked in the streptococcal background, but when the cloned *tetL* determinant is tested in *E. coli*, it can be shown that *tetL* specifies an energy-dependent efflux system (V. Burdett and S. B. Levy, upublished data).

Moreover, it is shown here that streptococci containing *tetM* or *tetN* possess a mechanism of tetracycline resistance in which transport of the antibiotic is unaffected by the presence of these resistance determinants. Tetracycline is accumulated at the same rate and to the same extent as it is in sensitive cells. Furthermore, the antibiotic recovered from inside the cells appears to be unmodified, and drug which has been incubated with extracts retains its biological activity. However, when cell-free extracts were tested in an in vitro protein-synthesizing system, it was found that protein synthesis in extracts prepared from cells carrying *tetM* or *tetN* was resistant to tetracycline, and this resistance could be increased 10-fold by growth in the presence of tetracycline. Inducibility in this system was difficult to determine by the more usual approaches, such as growth challenge experiments or by looking for increased resistance levels after exposure to antibiotic, and these have not been useful in evaluating inducibility in streptococci.

There are several possible explanations for the resistance of in vitro protein synthesis to tetracycline seen here. One is that resistance reflects functional alteration of the ribosome with respect to RNA or protein constituents. Alternatively, tetracycline could be sequestered by a tetracycline-binding protein within the cell, which prevents subsequent interaction of the drug with its ribosomal target. In this light it is interesting to note that in two organisms which produce tetracycline antibiotics, *Streptomyces aureofaciens* (13) and *Streptomyces rimosus* (18), salt-washed ribosomes were found to be sensitive to tetracycline in vitro, and resistance within these cells appears to be due to a ribosome-associated factor which has not been characterized. Experiments are

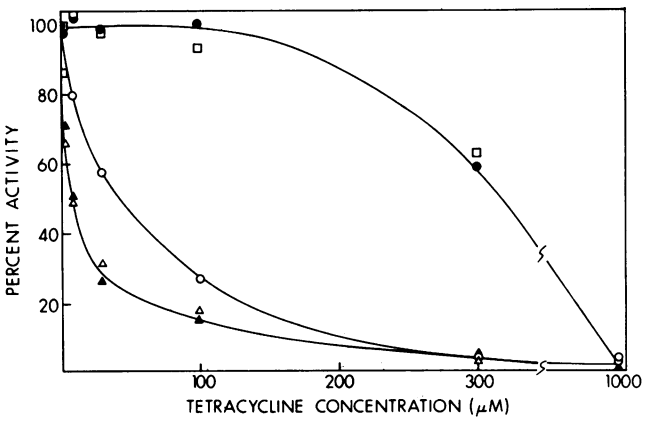

FIG. 6. Effect of tetracycline on in vitro protein synthesis. The ability of extracts derived from all cell types to synthesize protein in vitro was determined as described in Materials and Methods. Extracts were prepared from ATCC 9790r(pAM211) (*tetM*) (□) and ATCC 9790r(pMV120) (*tetN*) (●) grown in the presence of 20 μM tetracycline as well as ATCC 9790r (▲), ATCC 9790r(pMV158) (*tetL*) (△), and ATCC 9790r(pMV120) (○) grown in the absence of drug. Activities are depicted relative to the same extract assayed without tetracycline; all extracts tested synthesized protein to the same extent. Extracts prepared in this manner contained less than 0.2 CFU per assay. Incorporation of label in a typical assay ranged from 6,000 to 14,000 cpm over background.

currently under way to determine whether *tetM* or *tetN* shares sequences in common with *S. rimosus* or *S. aureofaciens* and to further characterize the nature of the streptococcal tetracycline resistance mediated by *tetM* and *tetN*.

ACKNOWLEDGMENTS

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