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Phenotypic Suppression in *Escherichia coli* by Chloramphenicol and Other Reversible Inhibitors of the Ribosome

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Antibiotics that interfere reversibly with various aspects of ribosomal function (chloramphenicol, tetracycline, erythromycin, and spectinomycin) are shown to antagonize, at barely inhibitory concentrations, the inhibitory effect of low concentrations of streptomycin (SM) on the growth of *Escherichia coli*. Paradoxically, these compounds can also replace SM in supporting the growth of conditionally SM-dependent mutants. Chloramphenicol produced about as much phenotypic suppression as SM in SM-sensitive strains, but less than that attainable with high concentrations of SM in resistant strains. The antagonism to SM inhibition and the phenotypic suppression appear to be specific for those growth inhibitors that act on the ribosome. Since inhibitors of the 50*S* subunit of the ribosome (chloramphenicol, erythromycin) are as active as inhibitors of the 30*S* subunit, it is suggested that phenotypic suppression by borderline concentrations of ribosome inhibitors does not necessarily depend on an alteration of the recognition region of the ribosome. Alternatively, partial inhibition of the ribosomes might change the environment in a way that would influence the frequency of misreading. Phenotypic suppression by a low concentration of SM as well as by chloramphenicol was found to depend on the presence of a trace of the required growth factor.

The well-known ability of reversible inhibitors of the ribosome, such as chloramphenicol (CA), to antagonize the bactericidal action of streptomycin (SM; references 1, 18, 23) seemed to be explained by the suggestion that this killing results from the production of abnormal protein (8, 14). This bactericidal mechanism, however, appears to be eliminated by the finding that the bactericidal and the misreading effects of the drug can be dissociated from each other in several ways (reviewed in 9); in particular, puromycin prevents protein formation and yet permits rapid killing (34, 35). An alternative mechanism is suggested by the observation that actinomycin D, in contrast, prevents killing (*unpublished observations*). Since this drug causes the ribosomes to be inactive for lack of messenger ribonucleic acid (RNA), whereas puromycin permits ribosomal activity, the irreversible effect of SM appears to require active engagement of the ribosomes in their cycle of protein synthesis.

Accordingly, the antagonism of ribosomal inhibitors to the lethal action of SM evidently depends on their ability to block the ribosome cycle. Some additional explanation, however, seems to be required for two further effects, which will be described in the present paper. At borderline, partly inhibitory concentrations, these drugs can *antagonize the inhibition* of the growth of SM-sensitive *Escherichia coli* cells by low concentrations of SM; and they can also *replace* SM in causing phenotypic suppression of conditionally SM-dependent (CSD) mutants. Possible mechanisms will be discussed.

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MATERIALS AND METHODS

Organisms. Wild-type *E. coli* strains B, W, and ML35 were used. CSD auxotrophic derivatives of an SM-resistant and an SM-sensitive mutant of *E. coli* B (14, 16) were kindly provided by L. Gorini and E. Kataja. Non-CSD amino acid auxotrophs had been isolated earlier in this laboratory from *E. coli* W.

Media and growth. Minimal medium A (10) was supplemented with 0.1% glucose (for overnight cultures) or 0.2% glucose (for studies with exponentially growing cultures), and with required amino acids at 100 μg/ml. Solid minimal medium contained 0.5%

glucose and 1.5% Difco agar. Incubation was at 37 C. Viable cell counts were determined in tryptic digest-agar pour plates, incubated for 16 hr.

Protein synthesis. Protein synthesis was measured as the incorporation of ^{14}C-leucine (0.65 μc/mg, 40 μg/ml; Schwarz Bio Research Inc.) Samples of the culture were transferred at intervals into an equal volume of 10% trichloroacetic acid. They were filtered, washed, dried, and counted in a Nuclear-Chicago gas-flow counter.

RESULTS

Antagonism of SM Inhibition by Reversible Inhibitors of the Ribosome

Effect of SM and CA on protein synthesis and on killing. When a moderate concentration of SM (20 μg/ml) was added to *E. coli* ML35 growing exponentially in minimal medium, protein synthesis soon became linear and then ceased at 60 min (Fig. 1B); killing began after 30 min and continued at a high rate for more than 1 hr (Fig. 1A). CA at 1 μg/ml slowed multiplication and protein synthesis, and it markedly delayed killing by SM (Fig. 1A and B). Moreover, although protein synthesis was slower with the mixture of CA and SM than with either alone, it continued longer and eventually became greater than with SM alone. This more prolonged synthesis of protein, which did not persist much beyond the 130 min shown in Fig. 1B, may merely reflect the continued activity of cells in which irreversible inhibition by SM was delayed by CA. Nevertheless, these findings led us to study the interaction of SM and CA in solid media, with unexpected results.

Stimulation of prolonged growth in solid media containing SM: effect of CA. Conventional filter paper disc assays with CA (in 0.01 to 0.02 ml of water) were carried out with pour plates heavily seeded with cells of *E. coli* B (ca. 10^7/ml) and containing the minimal concentration of SM required to prevent the formation of visible microcolonies (3 to 5 μg/ml). After 2 days of incubation, the discs containing 1 or 2 μg of CA were surrounded by a zone of heavy growth, while a larger amount (4 μg) yielded a zone of inhibition within a ring of similar stimulation (Fig. 2). The scattered colonies seen in the background (Fig. 2) reflect the high frequency of mutation to the slight resistance required to overcome the low concentration of SM. Similar results were obtained with strains W and ML35 of *E. coli* (Table 1).

The antagonism between CA and SM was restricted to barely inhibitory concentrations, and it could not be demonstrated in the reverse direction: with an SM-resistant (*str*^{r}) strain even 500 μg of SM per ml failed to reverse the effect of a minimal inhibitory concentration of CA.

Other drugs. Since the effectiveness of SM is quite responsive to various metabolic changes,

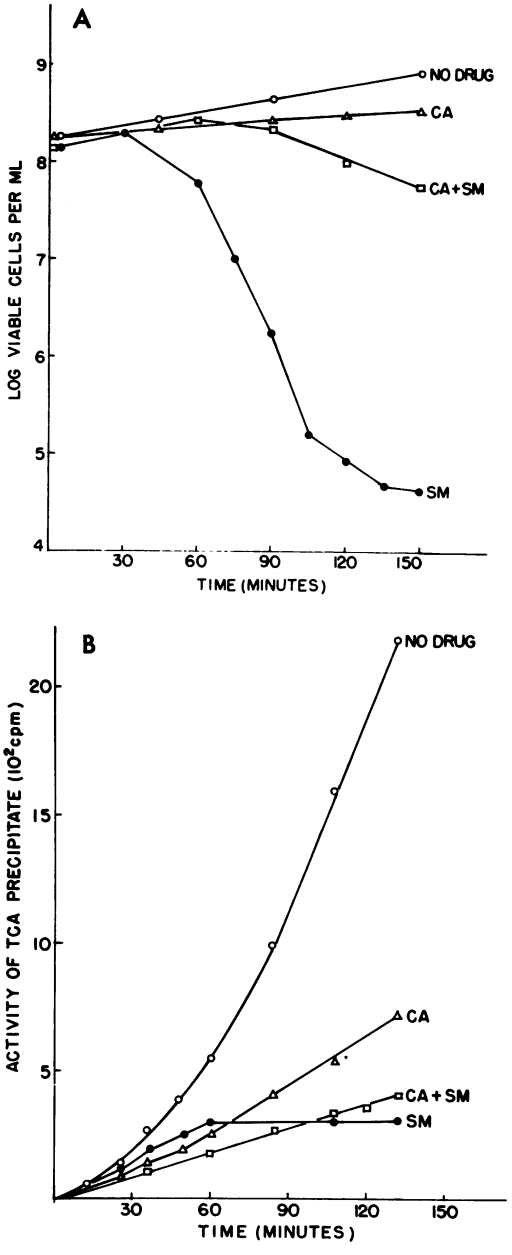

FIG. 1. *Effect of low concentration of CA on growth and protein synthesis in E. coli, and on response to SM. To a culture of E. coli ML 35, growing exponentially in minimal medium + 0.5% glycerol, was added ^{14}C-leucine plus CA (1 μg/ml) and SM (20 μg/ml) as indicated. Samples were removed at intervals for determination of viable number and incorporated radioactivity.*

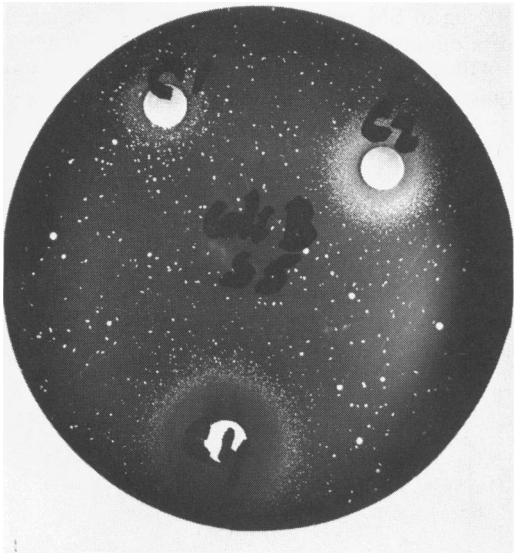

FIG. 2. *Growth of E. coli B in the presence of SM plus CA. CA (1, 2, or 4 μg) was applied to filter paper discs on solid minimal medium containing 5 μg of SM and 10^7 cells/ml. Visible growth around the discs appeared after 2 days of incubation and became heavier during 2 additional days; photographed at 4 days.*

such as a shift from aerobiosis to anaerobiosis (3, 19), it seemed possible that CA could be antagonizing SM only indirectly, as a result of depressing the growth rate. Accordingly, other bacteriostatic drugs were similarly tested against borderline concentrations of SM. Similar, though weaker, antagonism was produced by three additional drugs that inhibit protein synthesis reversibly on the ribosome: tetracycline, erythromycin, and spectinomycin (Table 1). Sodium azide, which inhibits electron transport, also antagonized inhibition by SM. In contrast, no such effect could be seen with several other drugs: novobiocin, which reversibly inhibits DNA and RNA synthesis (25, 26); sulfadiazine, which inhibits (via folic acid) the biosynthesis of various nucleotides and amino acids; or puromycin or 5-methyltryptophan, which inhibit protein synthesis by mechanisms that do not involve inhibition of a ribosomal function. Antagonism to inhibition of growth by SM is thus apparently restricted to drugs that inhibit the ribosome or that shift metabolism to an anaerobic pattern.

STIMULATION OF CONDITIONALLY SM-DEPENDENT MUTANTS BY REVERSIBLE INHIBITORS OF PROTEIN SYNTHESIS

The above results suggested that CA might directly antagonize the inhibitory action of SM on sensitive ribosomes. To see whether CA might also antagonize the equally characteristic action of SM in promoting misreading of the genetic code, an *str*^{r}, CSD strain was tested in pour plates. No antagonism was seen; on the contrary, even when tested without SM, 2 to 4 μg of CA on a disc elicited a growth response (around a zone of inhibition) which appeared rather slowly, like the response to 5 μg of SM (Fig. 3). The response to an excess of SM (100 μg) was faster as well as heavier, but as with most CSD mutants (14), it was still slower than the response to the required amino acid.

Requirement for a trace of end product in the response to SM or to CA. In the experiment of Fig. 3, the inoculum was 0.1 ml of an unwashed

TABLE 1. *Antagonism of various compounds to the inhibition of growth of wild-type E. coli by SM*[a]

Compound	Amt	SM in plate	Strain	Visible growth
	μg/disc	μg/ml		
CA	1	5	ML35	+ (1)[b]
	1	4	W	+ (2)
Spectinomycin	20	4	ML35	+ (1)
	20	3	W	+ (3)
Erythromycin	20	4	ML35	+ (2)
	50	3	W	+ (3)
Tetracycline	3	4	ML35	+ (3)
Novobiocin	50	4	ML35	−
	100	3	W	−
Sulfadiazine	5	5	ML35	−
	5	4	W	−
5-Methyltryptophan	0.25	5	ML35	−
		4	W	−
Puromycin	10, 40	5	ML35	−
	10	4	W	−
Sodium azide	50	5	ML35	+ (1)
	50	4	W	+ (1)

[a] An overnight culture grown in minimal medium was plated at 10^5 cells/ml in the same medium solidified with 1.5% agar. The medium contained SM at the concentration noted. The various drugs were applied on filter paper discs on the surface, in an amount that gave a small zone of inhibition on an SM-free plate. The plates were incubated and growth was checked daily for 4 days.

[b] Numbers in parentheses indicate days when growth was first observed.

culture of a CSD methionine auxotroph, grown in a medium containing an excess of methionine. Much lighter inocula (10^{-3} to 10^{-4} ml), in contrast, failed to respond to CA. Since the smaller inocula contained less free methionine as well as fewer cells, washed cells were tested. Even with heavy inocula, such cells did not respond to CA unless the medium was supplemented with a trace (0.05 μg/ml) of the required amino acid (Table 2). Measurements of growth response in liquid medium showed that this supplement would support only a doubling of the largest inoculum (10^7 cells/ml) used in Table 2. It is evident that traces of growth factor in the inoculum have an important role in the test for phenotypic suppression.

A similar requirement for "primer" amino acid was observed (*see* Table 3) with a number of other mutants but not with three lysine auxotrophs that were incompletely blocked (i.e., that grew in liquid minimal medium with a doubling time of 7 hr and yielded visible growth on solid minimal medium within 4 to 5 days). This "leakiness" evidently provides the background growth required for weak phenotypic suppression.

With a small amount of SM, phenotypic suppression was found to depend similarly on a small supply of end product (Table 2). However, 500 μg of SM elicited a response even in the absence of end product, though considerably more slowly than in its presence.

Response of various str^r and str^s (SM-sensitive) auxotrophs to SM and to CA. Seventeen randomly chosen CSD amino acid auxotrophs, derived from an *str^r* mutant of *E. coli* B (14), were similarly tested. All but one of these strains responded to a small amount of CA or of SM (Table 3). Moreover, distinct and reproducible differences were seen in the relative response of various mutants to the two drugs. Thus, CSD-leu-2 and CSD-leu-5, which could be shown to be "leaky," responded to SM even without amino acid supplementation, but responded poorly or not de-

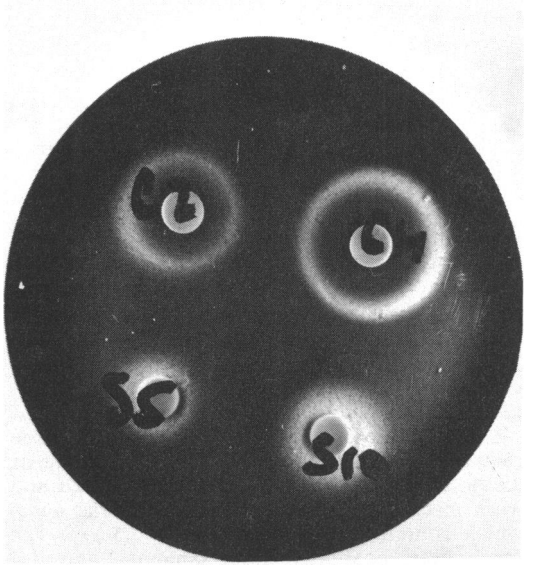

FIG. 3. *Growth response of an str^r CSD auxotroph to SM or CA. A culture of strain CSD-met-2 grown in minimal medium with methionine was plated at 10^7 cells/ml in minimal medium. Drugs applied to discs: CA, 2 and 4 μg (designated as C2 and C4); SM, 5 and 10 μg (designated as S5 and S10). Photographed after 2 days of incubation.*

TABLE 2. *Effect of trace of methionine (primer) on growth response of washed cells of CSD-met-2 to low concentrations of CA or SM*[a]

Methionine in medium (μg/ml)	Growth response					
	10^7 cells/ml		10^6 cells/ml		10^4 cells/ml	
	CA	SM	CA	SM	CA	SM
0.001	−	−	−	−	−	−
0.005	−	±	−	±	−	−
0.01	−	±	+	+	±	±
0.05	+	+	+	+	+	+
0.1	+ (17)[b]	+ (15)	+ (24)	+ (16)	+ (28)	+ (19)
1.0	+ (23)	+ (17)	+ (31)	+ (19)	+ (36)	+ (19)

[a] Cells of an overnight culture of *str^r* strain CSD-met-2 were washed with minimal medium and poured in agar-minimal medium supplemented with methionine as indicated. CA (4 μg) or SM (5 μg) was applied to filter paper discs on the surface. The plates were incubated and growth responses were recorded at 3 days: −, no response; +, easily visible response; ±, faint response.

[b] Numbers in parentheses indicate the diameter of the growth zone (mm) after 6 days of incubation. Note that the zone was larger with lower concentrations of cells, as would be expected if the diffusing drug was largely taken up by the cells.

TABLE 3. *Stimulation of growth of CSD mutants by low concentration of CA or SM*[a]

Mutant	Growth response			
	CA		SM	
	Amino acid absent	Amino acid present	Amino acid absent	Amino acid present
SM-resistant				
CSD-lys-1,2,3	+	+	+	+
CSD-leu-1,4	−	+	−	+
CSD-leu-2	−	−	+	+
CSD-leu-5	−	±	+	+
CSD-met-1,2,7,10	−	+	−	+
CSD-met-12	−	+	−	−
CSD-met-13	−	+	−	±
CSD-his-1,2,3	−	+	−	+
CSD-B4S-7-arg⁻	−	−	−	−
SM-sensitive				
leu^{CSD}-1,2,3,4	−	+	−	+
lys^{CSD}-5	−	+	−	+
B40-arg^{CSD}-1	+	+	+	+

[a] Plates were prepared as in Table 2, with the required amino acid present where indicated at 0.1 or 1.0 μg/ml. The plates were incubated and were checked daily for growth around the discs, which contained CA (4 μg) or SM (5 μg). The results were qualitatively the same at 2 and at 4 days.

tectably to CA, even with such supplementation. In contrast, CSD-met-12 responded to CA but not to SM; CSD-met-2 responded to either but more heavily to CA. Finally, arginine auxotroph CSD-B4S-7 did not respond to either SM (15) or CA under these conditions, although it did respond to a high concentration of SM (14).

At low concentrations, SM can also cause phenotypic suppression in certain auxotrophs of *str*^{s} strains (16). Six such mutants were tested with CA, and all responded (Table 3). The responses were equally strong with either drug, in the low concentrations that could be tolerated. A detectable response required primer amino acid with all but strain arg^{CSD}-B40-1. In the absence of primer, this exception responded more strongly to CA than to SM.

It has been reported (14, 16) that only a small fraction (<1%) of all auxotrophs exhibit phenotypic suppression by SM. The response to CA seems to be restricted to the same set of mutants. Thus, when 10 randomly chosen amino acid auxotrophs of *str*^{s} *E. coli* W were tested, none responded to CA (or to SM).

Effect of other compounds. Tetracycline, erythromycin, and spectinomycin, which (like CA) can antagonize the inhibition of the growth of sensitive strains by borderline concentrations of SM (Table 1), were also found to stimulate the growth of over half of the CSD mutants tested (Table 4). The responses, however, were generally weaker than to CA. Some strain specificity could be seen, just as in the earlier comparison of CA and a small amount of SM (Table 3); CSD-his-3 responded to tetracycline and to erythromycin but not to spectinomycin, whereas the opposite was true of CSD-his-1 (Table 4). Puromycin, which had not antagonized SM inhibition (Table 1), very weakly stimulated growth of some strains; this drug acts on the ribosome, but not by blocking its cycle.

Several derivatives of CA were also tested, at 100 μg per disc, with those mutants that had proved most responsive to CA: CSD-lys-1, CSD-lys-2, CSD-lys-3, and CSD-met-1. With all four mutants, growth was stimulated by the derivatives that inhibited growth of the wild type (CA with a monochloroacetyl or a trifluoroacetyl group substituted for the dichloroacetyl group), but not by the compounds that did not inhibit wild-type growth (CA without the dichloroacetamido group, CA with the nitro replaced by an amino group, and the L-*erythro* isomer of CA).

To determine whether the response is secondary to slowing of growth from any cause or is specific for ribosome inhibitors, several other kinds of growth inhibitors were tested, as in Table 3, in amounts that would give a small zone of inhibition in complete medium. Sodium azide (50 μg/disc) and 5-methyltrytophan (0.25 μg/disc) did not stimulate growth of any strains. Novobiocin (100 μg/disc) stimulated only the leaky CSD mutants (lys-1, lys-2, and lys-3), and more feebly than any of the ribosomal reagents.

Growth stimulation in liquid medium: enzyme assays. When studied in liquid medium, several completely blocked *str*^{r}-CSD strains exhibited no response to low concentrations of CA (1 μg/ml) or of SM (5 to 30 μg/ml). Stimulation was observed, however, with an incompletely blocked mutant, CSD-lys-2. In minimal medium supplemented with SM (30 μg/ml), this strain grew at the normal rate (doubling time 54 min; Fig. 4), just as when supplemented with the required end product; without supplementation, it grew at first one-third as fast and, after several hours, more slowly. It is seen that CA at 1 μg/ml also stimulated growth, but much more weakly and after a considerable lag.

Since SM at a high concentration can demonstrably restore synthesis of an active form of a mutant enzyme in CSD mutants (13, 14), similar restoration was sought in a mutant suppressed by CA in liquid medium. In the experiment of Fig. 4,

TABLE 4. *Stimulation of growth of CSD mutants by various bacteriostatic drugs*[a]

Mutant[b]	Growth response							
	Tetracycline (2 μg/disc)		Erythromycin (100 μg/disc)		Spectinomycin (10 μg/disc)		Puromycin (40 μg/disc)	
	Amino acid absent	Amino acid present	Amino acid absent	Amino acid present	Amino acid absent	Amino acid present	Amino acid absent	Amino acid present
SM-resistant								
CSD-lys-1,3	+	+	+	+	+	+	+	+
CSD-lys-2	+	+	−	+	+	+	−	+
CSD-met-2,7	−	+	−	−	−	−	−	−
CSD-met-10	−	+	−	−	−	−	−	+
CSD-met-13	−	+	−	−	−	+	−	−
CSD-leu-1	−	−	−	−	−	±	−	−
CSD-leu-4	−	−	−	+	−	±	−	−
CSD-his-1	−	−	−	−	−	+	−	−
CSD-his-3	−	+	−	+	−	−	−	±
SM-sensitive								
leu^{CSD}-1,2,3	NT	NT	NT	NT	−	−	−	+

[a] Plates were prepared as in Table 2 (10^7 cells/ml), with the required amino acid added, where indicated, at 0.1 or 1.0 μg/ml. Each drug was applied in an amount that gave a small zone of inhibition of growth in complete medium. The growth response was recorded after 2 days of incubation; after 2 days more, no qualitative change was seen.

[b] All the mutants of Table 3 were tested, except that the six *str*[s] strains were not tested (NT) with tetracycline or erythromycin. The mutants of Table 3 not noted here did not respond to any of the drugs under the conditions of the test.

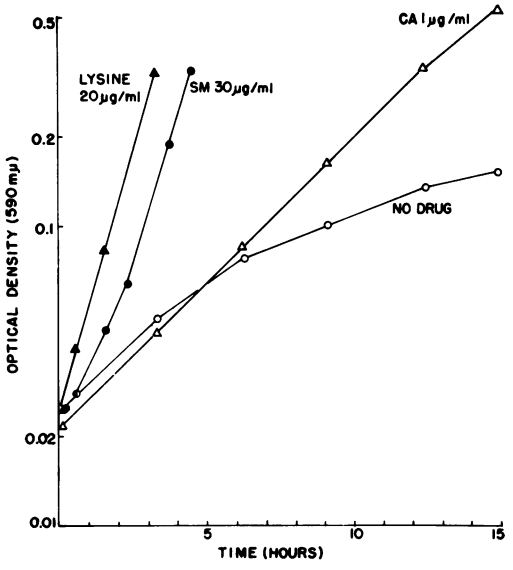

FIG. 4. *Growth response of an incompletely blocked str*[r] *auxotroph in liquid medium. Cells of strain CSD-lys-2, growing exponentially with shaking in minimal medium with lysine, were washed with minimal medium and were further incubated in this medium supplemented as indicated. After 15 hr, the cultures were shown to be free of lysine-independent revertants.*

activity of the defective enzyme, diaminopimelate decarboxylase, was detectable in extracts when the cells were grown in the presence of a moderately high concentration of SM (30 μg/ml), but not when the cells were grown (more slowly) in the presence of 1 μg of CA per ml.

Uniformity of response of population. Because the stimulation of CSD mutants by CA could be seen well only with prolonged incubation of heavily inoculated media, conceivably only a fraction of the population selected for increased drug resistance might be responding. This possibility was eliminated by showing that cells of *str*[r] mutant CSD-lys-3 yielded equal numbers of colonies on the surface of solid minimal medium containing CA (1 μg/ml), SM (10 μg/ml), or lysine, whereas, on minimal medium, tiny colonies of this leaky mutant appeared in smaller numbers (<10%).

DISCUSSION

We have shown that, at borderline (i.e., slightly inhibitory) concentrations, several reversible inhibitors of the ribosome (CA, tetracycline, erythromycin, and spectinomycin) can antagonize the inhibitory effect of low concentrations of SM on the growth of *str*[s] strains of *E. coli* in solid medium. Moreover, these compounds can also replace SM as a phenotypic suppressor, supporting the growth of CSD mutants. With most CSD

strains (*str*^{s} or *str*^{r}), growth was about as rapid with CA as it was with SM in *str*^{s} strains; with *str*^{r} strains, SM at higher concentrations produced more pronounced phenotypic suppression. Suppression by CA was shown to involve growth of all the cells of a culture rather than selection of resistant mutants.

It is evident that the test used in this work (auxanography in solid medium) can detect an extremely low degree of suppression. In liquid medium, in contrast, CA detectably stimulated growth of only one leaky *str*^{r} strain (Fig. 4), among several CSD mutants that responded in solid medium. Moreover, in the CA-stimulated cells, the activity of the mutant enzyme (diaminopimelate decarboxylase) was too low to be detected in extracts, whereas, with SM at 30 μg/ml, which stimulated faster growth, restoration of mutant enzyme activity was readily detected.

The antagonism to SM appears to be specific for inhibitors that act on the ribosome; no effect was obtained with borderline concentrations of several compounds that slow growth in other ways, including sulfonamides, 5-methyltryptophan (which blocks the synthesis of tryptophan; reference 22), novobiocin (25, 26), and puromycin. The specificity of the phenotypic suppression was similar, except that puromycin and novobiocin very weakly stimulated the growth of some incompletely blocked CSD mutants.

It seems very unlikely that reversible inhibitors produce the effects described here by being bound to the same ribosomal site as SM; the effective compounds differ widely in structure from SM, and they also appear to act on different parts of the ribosome. Thus, although spectinomycin acts, like SM (6), on the 30*S* subunit (7), the mechanism is quite different (2), and mutations to SM resistance and those to spectinomycin resistance alter different molecules in the 30*S* subunit (27). Tetracycline also appears to act on the 30*S* subunit, for more of it is bound tightly by this subunit than by the 50*S* subunit (4, 11, 12, 20), and it inhibits the binding of aminoacyl transfer RNA to 30*S* subunits (29) as well as to ribosomes (17, 28). However, it clearly differs from SM in its mechanism of action (which is not bactericidal) and hence in its binding site. CA and erythromycin act at more remote sites, since they bind specifically to the 50*S* subunit (31, 33); moreover, CA appears to block directly the peptide-forming step (5, 32), and erythromycin the translocation step (5).

Although the various reversible inhibitors thus bind to different sites on the ribosome, the resulting changes in these sites might conceivably have a similar allosteric effect on the SM-binding site, resulting in both a decrease in affinity for SM and a stimulation of misreading. Such a direct mechanism, involving increased ribosomal ambiguity, is favored by the observation that various reversible inhibitors of the ribosome differ somewhat from each other (Table 4), as well as from SM (Table 3), in their relative activity in supporting growth of different CSD mutants. In similar auxanographic tests, a neomycin-dependent mutant of *E. coli* has been reported to respond to macrolides but not to CA or tetracycline (24).

Nevertheless, it would seem remarkable if the SM-binding site (in the recognition region of the 30*S* subunit) could be distorted by inhibitors that bind to the 50*S* subunit (CA, erythromycin) just as by those that bind to the 30*S* subunit (tetracycline, spectinomycin). Hence, even though such distortion is almost certainly the cause of phenotypic suppression by those ribosomal reagents that do not inhibit the ribosomes (e.g., SM acting on *str*^{r} cells), those that do inhibit might suppress by a more indirect mechanism; for if some ribosomes are blocked, or if all are slowed, the remaining ribosomal activity will occur in an altered environment. For example, CA causes an increase in the charging of transfer RNA in cells (21), which could promote suppression by influencing the competition of various species of aminoacyl-transfer RNA with each other or with a polypeptide-releasing protein. Moreover, these considerations may apply not only to reversible ribosomal inhibitors, but also to SM acting at sublethal concentrations on *str*^{s} CSD cells. However, such an indirect mechanism might not be able alone to account for the relatively high levels of restoration of mutant enzyme activity that have been observed in some *str*^{s} strains (13).

An indirect mechanism could also account for the antagonism of borderline concentrations of reversible ribosome inhibitors to the inhibition of growth by SM (Fig. 2). For example, cells partly inhibited by CA develop an increased content of RNA (30), which might raise the threshold of growth inhibition by SM, since this drug complexes readily with RNA.

In the course of this work we observed that the suppression of CSD amino acid auxotrophs by CA, or by SM at low concentrations, requires a small amount of "primer" amino acid (except with incompletely blocked mutants, which can provide their own primer). Since the supplement is effective in an amount (Table 2) that can support only a doubling of the inoculated cells, it cannot be acting simply by adding its limited yield of growth to that supported by the drug. Evidently the frequency of "correction" of a mutant protein, which is less than 1% for a high con-

centration of SM (14), must be so low for CA that its effect on the low rate of translation supported by protein turnover fails to yield enough active enzyme to initiate growth. During the protein synthesis supported by the primer, however, the suppressor has an increased opportunity to cause the cell to make some active enzyme. Since the product of this enzyme should then be able to supplant the primer, growth understandably becomes self-perpetuating in the continuing presence of the suppressor. Hence, it seems desirable to include a trace of primer in tests for phenotypic suppression.

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