cells; the other factor, the inhibitor, interferes with the interaction of the activator and the cells. Both factors were prepared in cell-free form.

* This investigation was partially supported by grant G-12894 from the National Science Foundation.
† Aided by a grant for a postdoctoral fellowship from the American Cancer Society.
3 Hotchkiss, R. D., these PROCEEDINGS, 40, 49 (1954).
8 Tomasz, A., to be published.
9 Hotchkiss, R. D., unpublished data.
10 This effect therefore seems to be different from that of bacterial DNase which also appears at low levels in late culture filtrates. 4

PHENOTYPIC REPAIR BY STREPTOMYCIN OF DEFECTIVE GENOTYPES IN E. COLI*

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We have found a class of mutants of Escherichia coli which indicates the existence of a streptomycin-activated suppression mechanism. These mutants show a phenotype of conditional Sm dependence (CSD), for they are dependent on Sm for growth in minimal medium but do not require Sm in minimal medium supplemented with specific amino acids. The distribution of the CSD mutants among amino acid auxotrophs is sufficiently random to exclude the possibility that this action of Sm may be due to its participation as substrate or cofactor in some reaction in intermediary metabolism.

For two CSD-arginine auxotrophs (B4O<sup>SD</sup> and B4S-7<sup>SD</sup>), isolated some years ago by one of us, 1 it was found that ornithine-transcarbamylase (OTC), the enzyme converting ornithine to citrulline, was absent in cells grown with arginine or citrulline but was present in cells grown with Sm (with or without arginine). B4S-7<sup>SD</sup> was derived by a one-step mutation from B4S-7 which produces all the arginine enzymes at a high, derepressed level. 1a B4S-7<sup>SD</sup>, grown on arginine or citrulline, produces the same high level of all the enzymes (except OTC). It can thus be concluded that the appearance of OTC activity in B4S-7<sup>SD</sup> brought about by Sm is not due to the release of any known cytoplasmic repression. Furthermore, it was found that addition of Sm directly to the enzyme assay mixture has no effect on OTC activity as measured by the standard assay procedure. Similarly, preincubation with and without Sm of the inactive enzyme preparation from cells grown with-
out Sm has no detectable activating effect. It is therefore considered unlikely that Sm acts to stabilize or activate an otherwise inactive enzyme in the CSD mutants.

The purpose of this paper is to analyze the genetic elements determining the CSD phenotype and their relation to the Sm\textsuperscript{r} mutation. It appears that this new class of mutants may be regarded as a bacterial counterpart of the class of amber mutants\textsuperscript{2} in bacteriophages.

**Origin, Mutagenic Treatment, and Selection of the CSD Mutants.**—CSD mutants have been obtained from *E. coli* strain B (wild type) and from strain K12 (mutant 2320, F\textsuperscript{−}B\textsubscript{−}−i−O\textsuperscript{−}Sm\textsuperscript{r}, from the collection of Dr. A. Pardee). All CSD mutants (except the CSD-arg mutants whose origin has already been described\textsuperscript{1}) have been isolated with the following procedure: (1) mutation to Sm resistance, and (2) mutation to a Sm-suppressible defect in a structural gene (Enz\textsuperscript{SD}). The Sm\textsuperscript{r} mutation was a spontaneous one in B and, to the best of our knowledge, in K2320. The subsequent Enz\textsuperscript{SD} mutations resulting in the CSD phenotype were obtained by nitrosoguanidine mutagenic treatment.

The isolation from Sm\textsuperscript{r} B and K12 yielded 1 per cent of CSD mutants among the auxotrophic mutations observed (Table 1). In contrast several attempts to isolate CSD mutants in one step from Sm\textsuperscript{r} B and K12 have been unsuccessful.

**TABLE 1**

**Classes of CSD Mutants Which Have Been Isolated**

<table>
<thead>
<tr>
<th>Parent strain</th>
<th>Pathway affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>B wild type</td>
<td>Arginine (OTC); methionine (pre- and post-homocysteine); histidine (prehistidinol); phenylalanine; lysine, valine-isoleucine; leucine</td>
</tr>
<tr>
<td>B4S-7</td>
<td>Arginine (OTC)</td>
</tr>
<tr>
<td>K2320</td>
<td>Methionine; threonine; valine-isoleucine</td>
</tr>
</tbody>
</table>

Mutagenic treatment of Sm\textsuperscript{r} parent strains: washed cells from an exponentially growing culture were exposed for 31/2 hr to the action of N-methyl-N-nitroso-N-nitroguanidine (0.7 mg/ml) at 37\textdegree C in 0.2 M acetate buffer at pH 5.4.

CSD mutants were selected from the nitrosoguanidine-treated Sm\textsuperscript{r} parents by a modified penicillin selection procedure\textsuperscript{4} after intermediate growth in nutrient broth. The cells were exposed to penicillin in minimal medium A plus glucose\textsuperscript{3} and spread on nutrient agar plates. The CSD mutants were then identified by replica plating onto minimal and minimal plus Sm (500 \(\mu g/ml\)) medium. The colonies growing on minimal plus Sm, and on nutrient medium but not on minimal medium, were isolated and tested for their specific requirements in the absence of Sm.

**The Conditional Dependence of Cell Growth on Sm.**—Several mutants in each class differ in their frequency of reversion to prototrophy (ranging from 10\textsuperscript{−4} to 10\textsuperscript{−9}) or in their growth rates in Sm medium. In every case, however, the growth response to Sm is slower than to the specific requirement. This indicates that the repair by Sm is always only a partial one. Mutants that grow very slowly even on minimal agar, but are stimulated by either Sm or a specific nutrient, have also been isolated.

The growth rates and final yields of a meth\textsuperscript{SD} strain growing in liquid minimal medium with addition of methionine, or of different amounts of Sm, or of a mixture of both factors, are shown in Table 2. It is evident that Sm alone may replace methionine, whereas it does not further stimulate growth when it is added together with methionine. All cultures reach similar optical densities except in minimal medium without additions, where no growth occurs. The growth rate in Sm alone is 2.2 times slower than in methionine alone. Other CSD strains have yielded similar results except for the growth rates in Sm, which vary widely. The growth rate is near its maximum with a Sm concentration of 5 \(\mu g/ml\).
TABLE 2

EFFECT OF METHIONINE OR STREPTOMYCIN ON THE GROWTH RATE AND FINAL YIELD OF STRAIN B-methSD - 2

<table>
<thead>
<tr>
<th>Methionine (50 µg/ml)</th>
<th>Medium</th>
<th>No Sm</th>
<th>No Sm</th>
<th>Sm (500 µg/ml)</th>
<th>5</th>
<th>50</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Sm (no additions)</td>
<td></td>
<td>63</td>
<td>63</td>
<td>144</td>
<td>0.474</td>
<td>0.492</td>
<td>0.408</td>
</tr>
<tr>
<td>Division time (min)</td>
<td>0.014</td>
<td>0.540</td>
<td>0.450</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final yield*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Optical density at 490 nm (Beckman DU) reached after overnight growth at 37°C.
Minimal medium—medium A containing an amount of glucose which should allow the wild type to reach an optical density of ≈0.600. Inoculum—cells grown overnight in methionine medium were inoculated to o.d. = 0.010.

Growth in Sm versus Enzyme Level.—The level of OTC was measured (after toluene treatment) in cells of B4S-7SD grown under different conditions. Table 3 shows that this enzyme is practically absent from cells grown in arginine, whereas growth in the presence of Sm, either alone or added to arginine, permitted the formation of active OTC. The maximum level (about 0.5 units) was reached at Sm concentrations of 20 µg/ml or above. This level represents only 0.2 per cent of the OTC activity which would be produced in the presence of a derepressed OTC+ structural gene as in the parent B4S-7 (220 OTC units).

TABLE 3

LEVEL OF ORNITHINE TRANSCARBAMYLASE IN CELLS OF B4S-7SD GROWN UNDER DIFFERENT CONDITIONS

<table>
<thead>
<tr>
<th>Arginine alone</th>
<th>Arginine 100 µg/ml</th>
<th>Streptomycin alone (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg/ml</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>0.01 KB29</td>
<td>0.247</td>
<td>0.495</td>
</tr>
<tr>
<td>0.01</td>
<td>0.456</td>
<td>0.260</td>
</tr>
<tr>
<td>0.447</td>
<td>0.495</td>
<td>0.485</td>
</tr>
</tbody>
</table>

The figures are enzyme units (µg of citrulline formed per hour per mg dry weight of cells). The cultures are grown overnight in minimal medium plus additions specified in the table. The cells are toluenized and the enzyme is determined as previously described.7

Since the cells grown with Sm contain OTC, when washed B4O-SD cells pregrown in Sm medium, with or without arginine, are resuspended in minimal medium, they continue to grow for several generations at a rate close to that observed in Sm. In contrast, cells pregrown in arginine alone are unable to divide when transferred to minimal medium. These observations are consistent with the results of an experiment in which the decrease of OTC level per cell is measured during the residual growth of B4O-SD after transfer from a Sm (100 µg/ml) medium to minimal medium. It can be seen (Fig. 1) that the OTC formed during growth in Sm is diluted out during the residual growth in minimal medium, but during the first generation the dilution is not as rapid as might be expected in the absence of new synthesis. It therefore seems likely that intracellularly bound Sm can continue for some time to promote enzyme synthesis.

Genetic Recombination Experiments.—The two strains harboring an OTCSD mutation (B4O-SD and B4S-7SD) were compared with three independently obtained OTC- strains (B90, B96, and B109) in interrupted mating experiments. These strains used as recipients, were all Val+ and Sm-. The donor strain K10 (an Hfr K12 originally from Dr. L. Cavalli) was Val+ Sm+ and OTC+ (an arginine prototroph). This Hfr strain injects its markers in the following sequence: origin-T6-Lac-OTC-Leu-Threo-Val+Sm-His. The interrupted mating technique7 was used with the modifications already described1 for K12 X B hybrid crosses. By plating in mini-
mal medium plus valine (10^{-3} M) as a counterselector for K10, it is possible to determine the time at which arg^{+} recombinants begin to appear. For all the recipients the time of first transfer of the arg^{+} character was the same (22–23 min). This was reported as the time of entry of the OTC structural gene in B × K12 crosses. The OTC^{SD} mutation is therefore in, or very close to, the OTC structural gene.

In an ordinary mating experiment, between B4S-7^{SD} (OTC^{SD} Val^{+} Sm^{+} his^{-}) and K10 (OTC^{+} Val^{+} Sm^{+} his^{+}), his^{+} Val^{+} recombinants were selected. Of 240 recombinants 77 per cent were arg^{+} and 23 per cent were arg^{-}. (The arg^{SD} character appears as arg^{-} when tested in the absence of Sm.) The distribution of the Sm character was as follows: among the arg^{+} recombinants, the Sm^{+}:Sm^{+} ratio was 81:19, and among the arg^{-} recombinants the ratio was 73:27. The essentially equal distribution of the Sm character among arg^{+} and arg^{-} recombinants, demonstrates that the OTC^{SD} and the Sm^{+} characters are unlinked. All arg^{-} Sm^{+} recombinants were found to be CSD-arginine.

The CSD Phenotype and Suppressors.—Out of 4 independent Sm^{+} mutants of the arginine auxotroph B40 (which harbors the OTC^{SD} mutation^{1}), only 2 yield the CSD phenotype. This result indicates that the Sm^{+} mutation is directly involved in producing the CSD phenotype, rather than being merely a coincidental requirement for its testing with Sm. This result is not consistent with the possibility that Sm acts directly to stabilize or activate the protein produced by the Enz^{SD} gene, and suggests that its action is at the level of protein synthesis itself. Since we found that in Sm medium, some Sm^{+} mutations cause the phenotype of a given OTC^{SD} mutant to be OTC^{-}, whereas other Sm^{+} mutations permit an OTC^{+} phenotype to appear (and we know that the Sm^{+} and OTC loci are unlinked), the second type of Sm^{+} mutation is formally equivalent to a suppressor (a mutation which reverses the phenotype produced by a gene mapping at a different locus). Moreover, since in the absence of Sm this suppressor function is not expressed (the phenotype is OTC^{-}), we suggest the existence of a Sm-activated suppressor mechanism.

It is not known whether the Enz^{SD} mutations harbored by the CSD mutants are also suppressible by genetic suppressors mapping outside the enzyme structural gene. However, a mutation which is known to be suppressible by outside sup-
pressors has been examined for its ability to be suppressed phenotypically with Sm. The strain K2320 (which incidentally is the parent of our K12-CSD mutants) harbors a Lac-operator negative mutation (O°). It has been reported\(^a\) that mutations at several loci outside the Lac-operon are individually able to suppress the O° mutation. It now appears that this O° mutation may also be phenotypically suppressed by Sm to some degree. This can be demonstrated with independent tests for the lactose permease, and for lactose fermentation. It is known\(^b\) that melibiose enters the cell specifically via the lactose-permease mechanism although it is utilized by an enzyme other than β-galactosidase. Therefore, to detect lactose permease activity independently from β-galactosidase, cells of K2320 (Sm') were spread on agar containing melibiose as sole carbon source, with and without Sm (500 μg/ml). After overnight incubation at 37°, minute colonies appeared only on the melibiose plate containing Sm. To detect lactose fermentation, the cells were spread in the same way, on EMB agar with and without Sm. After 2 days' incubation, only the colonies on EMB plus Sm exhibited a core of pink color.

When a Sm', β-galactoside-permease negative mutant was tested in the same way, its phenotype was not influenced by the presence of Sm. This result rules out the possibility that Sm acts via an effect on the cell membrane, permitting the entry of melibiose independently of the permease. Therefore the slight phenotypic suppression of the Lac-operator negative mutation effected by Sm seems significant.

Our findings permit the interpretation of an earlier unpublished observation of L. Cavalli and E. Lederberg.\(^{10}\) A spontaneous "reversion" of a K12 gal− strain was isolated and shown to have a gal− Su+ genotype. Approximately 40 per cent of the Sm' mutants derived from the Su+ strain were phenotypically gal− except in the presence of Sm, when they appeared gal+. M. Meselson\(^{11}\) has recently made a similar observation in strain C600 of E. coli. This strain contains a suppressor which permits the growth of suppressor-sensitive mutants of phage lambda.\(^{12}\) It has been found that in the absence of Sm, some Sm' derivatives behave as Su−, except in the presence of Sm when they appear Su+.

The experiment with the K2320 Lac-operator negative mutant demonstrates that Sm can suppress a defect also suppressible by mutations mapping at a locus outside the Lac-operon and the Sm locus. The experiments of Cavalli and Lederberg and of Meselson confirm our finding that not all Sm' mutations permit the CSD phenotype to be expressed. In addition, they show that the occurrence of a mutation from Sm' to certain types of Sm' changes the sign of a Su gene from + to − in the absence of Sm. This observation excludes the possibility that the noncompetent Sm' mutations are mutations to Sm-impermeability.

Since not all Sm' mutations are competent when tested with a given suppressible defect, and yet the randomly chosen Sm' mutations of the B and K2320 strains were both competent to permit some CSD mutants, the possibility exists that for any given Sm' mutation, a class of genetic defects exists for which that Sm' is competent. This would imply some type of complementarity between the competent Sm' mutation and the suppressible defect.

**Conclusions.**—The results indicate that the CSD phenotype we have observed is the result of the concomitant expression of at least two genetic elements: a suppressible defect and a suppressor whose function is dependent on Sm (Su'sm'). Since not all Sm' mutations reveal this suppressor function when tested with a suppress-
GENETICS: GORINI AND KATAJA

possible defect, there are two possibilities. Either \((a)\) the locus of the suppressor is identical to the Sm\(^{-}\) locus and the suppressor mutation arises de novo via a single event at the Sm\(^{-}\) locus, or \((b)\) the suppressor mutation is present gratuitously in the parents of the CSD strains and, in addition to Sm, requires for its function a subsequent mutation at the Sm\(^{-}\) locus.

There is no evidence which excludes the possibility that the Sm-activated suppression mechanism may act at the level of transcription. However, since there is evidence that mutations at the Sm\(^{-}\) locus result in ribosomal alterations,\(^{13-16}\) we focus our attention on the level of translation from messenger to protein. In this context it should be pointed out that if the \(S_{\text{Sm}}^{+}\) and Sm\(^{-}\) loci are identical, this would imply that the ribosomal structure could influence the accuracy of the reading of the code during its translation.

If a Sm-suppressible defect involved a vital function (e.g., RNA polymerase, activating enzyme, transfer RNA) the mutant would fall into the category of classical Sm dependent mutants, i.e., it would require Sm for growth under all conditions.

Since in the CSD type of mutant one can control the expression of a vital function with an external agent (Sm), it offers the opportunity for mapping vital functions in bacteria (as do amber and sus mutants in bacteriophages). Temperature-sensitive mutants provide the same opportunity, but their usefulness is limited to the study of cistrons governing protein structure.

Summary.—A new class of mutants has been isolated, which are conditionally streptomycin-dependent (CSD), i.e., they require either a specific metabolite or streptomycin. One of these mutants (an arginine auxotroph) has been shown to possess a defective structural gene which is phenotypically corrected by streptomycin. Genetic studies of this strain provide evidence that its CSD phenotype results from the presence of a suppressible gene defect and a suppressor mechanism whose function is dependent on streptomycin.

The skillful technical assistance of Mrs. Frayda Oston and Mrs. Regine Weil, and the invaluable advice and discussion of Dr. W. Gilbert and Dr. B. D. Davis for the preparation of this manuscript are gratefully acknowledged.

The following abbreviations are used: Sm, streptomycin; CSD, conditional streptomycin-dependent; OTC, ornithine transcarbamylase; \(F\), fertility factor; \(B\), thiamine; \(i\), \(\beta\)-galactosidase control; \(o\), Lac-operator; \(Lac\), lactose operon; \(S_{\text{Sm}}^{+}\), streptomycin-activatable suppressor; nitrosoguanidine, N-methyl-N-nitroso-N-nitroguanidine; (K & K Laboratories).

\(^{*}\) This work was supported by a USPHS grant (AI-02011-06) from the National Institute of Allergy and Infectious Diseases, an American Cancer Society grant (E-226-B), and USPHS fellowship (5-F1-GM-19,085-02) from the National Institute of General Medical Sciences.


\(^{13}\) The enzymes of the arginine biosynthetic pathway in strain B although not repressible by arginine, have a low, partially repressed level. Strain B has therefore been designated \(R_{\text{arg}}^{-}R_{x}^{+}\). Mutants derived from \(B\), having completely derepressed enzyme levels, have been designated \(R_{\text{arg}}^{-}R_{x}^{-}\). The genotypes and phenotypes of the strains pertinent to the discussion of the CSD-arginine mutants are given below. Each strain was derived from wild-type B (Sm\(^{+}\)R_{arg}^-R_{x}^-OTC\(^{+}\)) by a series of one-step mutations imposed in the following order: B4O: SmR_{arg}-R_{x}^{+}OTC\(^{80}\) (phenotypically arg\(^{+}\)); B4O Sm\(^{+}\): SmR_{arg}^-R_{x}^-OTC\(^{80}\) (phenotypically arg\(^{-}\)); B4O 5\(^{80}\): SmR_{arg}^-R_{x}^-OTC\(^{80}\) (phenotypically arg\(^{-}\)); B4S-7\(^{80}\): SmR_{arg}^-R_{x}^-OTC\(^{80}\) (phenotypically CSD-arg).
THE ANTIGENICITY OF TROPOCOLLAGEN*

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Collagen is a structural protein which constitutes about 30 per cent by weight of all protein in the mammalian body. The collagen fiber itself is relatively insoluble, but various conditions of solvent, pH, ionic environment,1 and temperature2 may be used to obtain soluble fractions. Physical characterization of these soluble fractions, and X-ray and electron micrograph studies on precipitates obtained under specific conditions, have shown that the native collagen fiber is built up by the highly ordered aggregation of a fundamental unit termed the tropocollagen (TC) molecule,3–5 which is a stiff rod with a length of approximately 2800 Å and a molecular weight of about 300,000. The ~700 Å periodicity seen in electron micrographs of collagen fibrils is believed to reflect the aggregation of the tropocollagen monomers in a polarized quarter-stagger array.6

The internal structure of the TC molecule is characterized by three polypeptide chains wound in a triple helix.7 The basic structural unit is the α-chain—a polypeptide strand with a molecular weight of approximately 100,000.8 Two α-chains may be cross-linked to form a β-chain, and three to form a γ-chain.9, 10

The processes initiating and controlling the formation of a native-type fibril in vivo from TC monomers are unknown. It might proceed via the lateral aggregation of staggered monomers with a consequent progressive build-up of a filament, or it could proceed via the lateral aggregation of protofibrils which form initially by end-to-end polymerization of the monomers. It was demonstrated in this laboratory that sonic irradiation modifies the normal end-to-end interactions of the monomers, and it was proposed that TC molecules bear peptide "end-chains"