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INTRODUCTION

This thesis deals with genetic and biochemical studies on an extragenic suppressor of a mutation at the ssd locus in Escherichia coli K-12.

Suppression is a genetic term referring to the action of a secondary mutation that restores a wild type or pseudo wild type phenotype to a mutant organism without altering the primary mutation. A suppressed strain carries two mutations, the second modifying or cancelling out the action of the first, and thus "suppressing" the mutant phenotype.

The primary mutation, ssd, studied here is highly pleiotropic and is of particular interest because the explanation for the metabolic diversity of its various pleiotropic effects is not obvious. It is hoped that the study of the suppressor will help to elucidate the nature of the ssd mutation.

Section I

The Isolation, Characterization and Map Location of the SSD Mutation

In order to understand the experiments concerning a suppressor of the ssd mutation, a review of the nature of the ssd mutation in itself is required. The ssd mutation was isolated in a transduction experiment using strain MS845, carrying the equally pleiotropic wyb mutation (1), as donor and CU1008 as recipient using growth with serine as carbon source as a selective marker. The relationship between wyb and ssd is not yet well understood (2). The various phenotypic effects of ssd are:

1) inability to use succinate as carbon and energy source.
2) inability to grow anaerobically in rich or minimal medium.
3) decreased growth rate on glucose minimal medium.
4) a very high and noninducible l-serine deaminase activity.

All these effects are due to a single mutation which has been mapped unequivocally at 86 minutes in the E. coli linkage map between the rha and met B loci.

Section II

Indication of a Suppressor of the SSD Mutation

While doing the experiments designed to locate the ssd mutation, Morris (2) found preliminary evidence for the existence of a suppressor. He used the ssd mutant strain VE2-2 and a strain, MAF1/JC1553, that carries an F' episome #140 carrying DNA from 68 to 80 minutes. The strain VE2-2 when infected with episome could use succinate as carbon source though the ssd mutant itself cannot. Since F' episome carried DNA from 68 to 80 minutes only, it therefore cannot contribute a functional ssd gene (86 minutes). In that case, it must therefore carry a gene between 68 to 80 minutes which confers ability to use succinate on the ssd strain, i.e., by definition, a suppressor of ssd.

The experiments in this thesis are designed to confirm the existence of a suppressor and to study it.

Section III

Review on Genetic Suppression

In order to understand the nature of suppressor mutations a review on suppression is required. A mutant strain can be considered to have some altered mechanism, e.g. a faulty protein or a defective transport system. The suppressor mutation will restore the phenotype to normal. Suppressor mutations are usually considered in two classes, intragenic ("internal") meaning that the second mutation is in the same cistron as the first mutation, and extragenic ("external") meaning that the second mutation is in a cistron other than the one carrying the first mutation.

An intragenic suppressor mutation, i.e., a secondary change within the original mutant gene, could restore the normal phenotype due to a number of possible mechanisms depending on the nature of the primary mutation and how it affects the normal phenotype. A number of cases have been reviewed by Hartman and Roth (3) and some of these are discussed here.

Many cases of intragenic suppression have been reported where the amino-acid sequence of a gene is changed at some site due to a primary mutation that changes one of the three bases of a codon. A

suppressor mutation substitutes another base in the same codon thus leading to an amino acid sequence different from the normal one but the altered gene product is partially or completely functional. One example of this sort has been reported by Helinski and Yanofsky (4) in the E. coli tryptophan synthetase A gene. In this case the primary or the original mutation alters the triplet coding for residue 210 leading to a substitution of an arginine (AGA) in place of glycine (GGA) which produces an inactive enzyme protein. A suppressor mutation in the same codon changes the codon from AGA (Arginine) to UG^{u}_{c} (serine) and this sequence leads to the restoration of enzyme activity.

Another possible mechanism of intragenic suppression could be by a mutation that restores the active conformation of the mutated gene product. If the original mutation leads to an inactive conformation of a gene product a second mutation at another site could cause a change that restores the inactive conformation to an active one by some kind of interaction between two mutation sites. Abelson et al. (5) have reported many such cases in E. coli $t_a RNA^{tyr}$. Here the residue 2 normally pairs with residue 80. The primary mutation changes residue

2 from G → A making it unable to pair with residue 80 and the t RNA becomes temperature sensitive. A second mutation at site 80, from C → U, enables these two bases to pair again and thus restores the normal function of these t RNAs.

A mutation that causes a change in the initation site of the gene making it unable to initiate the translation, could be suppressed by a second site mutation in the initiator region of the gene creating a new initiator codon. Sherman et al. (6) have reported such a case in yeast iso-1-cytochrome c. The primary mutation causes loss of the initiator codon AUG (met) and an intragenic suppressor mutation creates another AUG codon within the initiator region, thus restoring translation.

Another mechanism of suppression was observed in cases where the original mutation is a frameshift mutation. The mutated gene carries either an addition (+1) or a deletion (-1) of one base. A second mutation which causes a deletion or an addition of one base will restore the normal reading frame. If this second mutation is sufficiently close to the first, the product made may be functional and the second mutation would then be a suppressor. Such a case of suppression by double frameshift mutation has been reported by

Berger et al. (7 , 8) in E. coli tryptophane synthetase A gene. The primary mutation is a frameshift mutation that shifts the reading frame in such a way that a nonsense codon is formed and therefore termination of translation occurs at that place. Another frameshift mutation shifts the reading frame back to normal restoring the normal protein.

If a mutation leads to a toxic or lethal gene product, an intragenic mutation that eliminates the initiator region or substitutes a nonsense mutation so that either no gene product is formed or an early termination of transcription or translation occurs, will be a case of suppression. Hartman and Roth (3) have reviewed such a case studied by Barnett et al. (9) in bacteriophage T4. The primary mutation in rIIB cistron leads to a polypeptide that is toxic to the growth of phage. An intragenic suppressor mutation that substitutes a nonsense codon leading to an early chain termination has been reported.

These are a few of the many cases of intragenic suppression. The suppressor of mutation studied in these thesis is not intragenic as it has been mapped quite far from the original mutation ssd.

The intergenic suppressor mutations are usually considered in two classes, direct (informational)

and indirect. A mutant strain can be considered to have some altered mechanism and suppressor will either restore the mechanism to normal (direct) or will alter its environment so that the altered structure functions normally or is by passed entirely (indirect).

The direct suppressors modify the amino acid sequence of the mutant gene product. Since these suppressors act directly at the transcription-translation level where the information is given, they are also called informational suppressors. Yanofsky and St. Lawrence in 1960 (10) and Benzer and Champe in 1962 (11) gave a model for molecular mechanism of suppression by suggesting that some of the suppression events could occur due to alteration in the translation of the genetic message. This means that the mutant gene is transcribed into a mutant m RNA but the suppressed strain possesses some mechanism that translates the altered region of m RNA into a normal product resulting in a functional protein. The point mutations that have been found to be suppressed externally by such a mechanism include missense mutations, (where the mutant codon codes for an incorrect amino acid), nonsense mutations (where the mutant codon specifies no amino acid) and frameshift mutations (where an addition or deletion of a base

shifts the normal reading frame of translation of the message). In the case of external suppression of the mutations that result in missense codons, the suppressed strains produce an altered t RNA that can substitute an acceptable amino acid at the side of mutant codon resulting in a gene product with functional sequence of amino acids. An example of this kind of suppression has been studied by Hill (12) in the E. coli tryptophane synthetase gene. The primary mutation changes a "sense" codon specifying glycine (GGA) into a missense codon specifying arginine (AGA). The suppressor produces $tRNA^{gly}$ that recognizes the codon for arginine thus placing glycine at the site of mutant codon and bringing the mutant phenotype back to wild type. Many other suppressor $tRNA^{gly}$ have been reported for this trp A mutation.

In case of extragenic suppression of the three nonsense codons, UAG, UAA and UGA, that specify no amino acid and lead to the termination of translation, the suppressed strain produces an altered t RNA that can read the mutant codon and places an amino acid at that site resulting in a fully or partially active complete polypeptide chain. The degree of suppression depends on the extent of

restoration of tertiary structure of the protein by substitution of that particular amino acid. An example of such a case has been reported by Kaplan (13) in E. coli where a mutated t RNA^{lys} can recognize either of the two nonsense codons, UAA and UAG.

In most of the cases the t RNAs that mediate the suppression have an altered anticodon which enables them to pair and read a missense or nonsense codon in the m RNA but in some cases these suppressor t RNAs carry the wild type anticodon and the alteration is at some place out side the anticodon region. Hirsh (14) has reported a UGA-suppressing t RNA^{trp} with a mutation in the stem of the D-loop and an unchanged anticodon.

The extragenic suppression of frameshift mutation has been reported by many workers. Riddle and Carbon (15) have reported the existence of t RNA^{gly} suppressing a frameshift mutation (+1) by having a 4-base anticodon CCCC.

An easy test for recognizing the nature of these extragenic direct suppressors has been developed based on the idea that the strain carrying the suppressor will be able to allow a phage, carrying a specific kind of suppressible mutation, to grow. This test

was performed on one of the strains (KL14) carrying the suppressor of ssd mutation. Phage strains carrying nonsense mutations (UAA, UGA or UAG) and a frameshift mutation were tested for growth on the strain KL14. None of these were suppressed by this strain and hence the ssd suppressor mutation (su^-) is not similar to these nonsense or frameshift suppressor.

There have been found some cases of direct suppressor mutations where suppression occurs without any alteration in the t RNAs. In these cases the t RNAs are normal and the suppressor mutation is in the gene that codes for one of the structural proteins of the ribosome. An example of such a suppressor mutation is in the ram gene which suppresses str A mutations (16). The primary mutation str A is in the gene that codes for 30 s ribosomal protein S12 and results in an inefficiency of suppression of nonsense and missense mutation due to an alteration in the structure of ribosome. The ram gene codes for another 30 s ribosomal protein S4. A mutation in ram changes the structure of ribosome and acts as a suppressor of str A (17).

There have been reported many cases of indirect suppression, i.e. where the mutant gene product is formed even in the suppressed strain and the suppressors permit the normal function of this mutant gene product. Macromolecules in general are

sensitive to changes in their physical environment such as temperature, pH, etc. For example, the activity of an enzyme can be altered by alteration in the pH or ionic concentrations or by the presence of various effector molecules that influence regulation, induction, inhibition, repression etc. Thus an adjustment of the intracellular milieu may compensate for the abnormality imposed by a mutant. Various cases of such a suppression mechanism have been reported by Hartman and Roth (3). Some of these reports are discussed here.

An example of such a case of indirect suppression has been reported by Suskind and Yanofsky (18) in Neurospora tryptophan synthetase gene trp A. The primary mutation results in an enzyme protein that is sensitive to inhibition by zinc. In the suppressed strain the intracellular location and concentration of Zn^{++} is altered resulting in a wild type phenotype.

Similar cases of indirect suppression are possible in cases where the mutant enzyme is sensitive to some metabolite and the suppressor changes the rate of of formation of that particular metabolite, thus altering its concentration to a degree to which the mutant enzyme is not sensitive. An example of this

kind has been reported by Jacobson and Jacobson and Grell in 1971 (44, 45) in Drosophila. In this case, the mutant produces an specific t RNA that has an inhibitory effect on the tryptophan pyrrolase enzyme. The suppressed strains have a mechanism to eliminate these specific mutated t RNAs thus releasing the inhibition imposed by these t RNAs on the enzyme.

If a mutation causes a change in the active conformation of the gene product, a second mutation that can restore the tertiary structure of the mutant gene product could bring about the suppression. Nomura (19) has reported such a case of suppression in a bacterial ribosomal mutant. In this case, two proteins S19 and S7 co-operate in binding r RNA during ribosomal assembly. The mutation in S19 alters the binding and maturation and another mutation in S7 brings back the normal binding properties.

Some cases of indirect suppression have been reported where the primary mutation produces a protein or metabolite which is toxic to the cell. The suppressor mutation eliminates the toxic protein or converts the toxic metabolite into a nontoxic product. An interesting example of such a suppression event has been found in E. coli mutants that accumulate phosphate esters of some sugar because of a

genetic block in the metabolism of that particular sugar. The phosphate esters of sugars are toxic when accumulated. A mutation that prevents the accumulation suppresses the toxic effect of mutant. For example, galactose-1-phosphate mutants accumulate the phosphate ester of galactose; a second mutation in galactokinase that blocks another step of galactose metabolism has been shown to prevent the toxic accumulation (20). Similarly, Englesberg et al. (21) has reported in E. coli the suppression of an l-arabinose mutation that causes toxic accumulation l-ribulose l-phosphate by mutation in the l-ribulokinase enzyme. Many similar examples have been reported by Hartman and Roth (3) in their review article on suppressors.

Another interesting case of indirect suppression has been studied by Guespin-Michel (22) in Bacillus. Here the primary mutation is pleiotropic and affects polymyxin sensitivity and sporulation in the bacteria. The mutant is sensitive to polymyxins and defective in sporulation. The suppressed strains partially restores the normal phenotype by making an alternate protein molecule that restores lost membrane function and affects catabolic repression but does not restore sporulation.

In the light of this information the possible mechanism of ssd suppressor will be discussed later.

The mechanism of suppression of the ssd mutation is not yet understood nor is the ssd mutation itself. However, a discussion and comparison of the nature of ssd suppression with known suppressors will be made later.

MATERIALS AND METHODS

Strains Used: All the bacterial strains used for this study are derivatives of E. coli K-12. Their designations and relevant characteristics are listed in Table 1. The bacteriophages used and their relevant characteristics are listed in Table 2.

Throughout this report the gene symbol su^+ stands for wild type and su^- stands for the strains carrying the suppressor activity.

Media Used:

Minimal medium: 0.54% K_2HPO_4, 1.26% KH_2PO_4, 0.2% $(NH_4)_2SO_4$, 0.2% $MgSO_4.7H_2O$ and 0.001% $CaCl_2$ at pH 6.4 in distilled water.

Luria broth: 0.5% yeast extract, 0.5% NaCl, 1% tryptone in water.

Luria broth agar plates: Luria broth and 2% agar.

Recombination by Conjugation: Conjugation experiments were done by the standard method described by Miller (23). Donor and recipient strains were precultured in Luria broth for about 18 hours at 37°C and subcultured in fresh media for ~ 2 hours. Controls were made by plating donor and recipient strains on selective media, i.e., minimal medium supplemented with maltose and nalidixic acid (MV mal nal). One ml of the culture of the

Table 1

List of Bacterial Strains and their Relevant Characteristics

Strain	Genotype[a] and/or Relevant Characteristics	Source
1) CU1008	*ilv A* deletion	L.S. Williams
2) VE2-2	*ssd* ; CU1008 derivative	This laboratory
3) MND-1	*mal A*, nal^R derivative of VE2-2.	This laboratory
4) DNR-1	Su^-, Mal A^+, *ssd* ; recombinant of MND-1 using KL14 as donor selecting Mal^+.	This work
5) DNR-2	Su^-, Mal A^+, *ssd* ; recombinant of MND-1 using KL228 as donor selecting Mal^+.	This work

Table 1 (Cont'd)

Strain	Genotype[a] and/or Relevant Characteristics	Source
6) DNT-1	Su^-, Mal A^+, ssd ; transductant of MND-1 using KL14 as donor selecting Mal^+.	This work
7) DNT-2	Su^-, Mal A^+, ssd ; transudctant of MND-1 using KL228 as donor selecting Mal^+.	This work
8) DNT-3	Su^-, Mal A^+, ssd ; transductant of MND-1 using KL228 as donor selecting Mal^+.	This work
9) P4X	met B , rel 1.	CGS # 4312
10) DNP-1	Ssd^-; transductant of P4X using DNR-1 as donor selecting Mal^+.	This work

Table 1 (Cont'd)

Strain	Genotype[a] and/or Relevant Characteristics	Source
11) DNCU-1	*ssd* ; transductant of CU1008 *met B* using DNT-2 as donor selecting Mal$^+$.	This work
12) CU1008 met B$^-$	*met B* derivative of CU1008.	This laboratory
13) DNCU-2	Ssd$^+$; transductant of CU1008 *met B* using DNT-2 as donor selecting Met$^+$.	This work
14) KL14	*thi-1*, *rel A1*, *λ*$^-$. P068 between *arg G*$_{68}$ and *met C*$_{64}$. *arg G* → *met C*	CGS # 4294
15) KL228	*thi-1*, *leu B6*, *gal-6*, *lac Y1* or *lac Z4*, *λ*$^-$, *sup E44*. P013 between *rbs*$_{83}$ and *ilv E*$_{84}$. *rbs* → *ilv E*	CGS # 4318

Table 1 (Cont'd)

Strain	Genotype[a] and/or Relevant Characteristics	Source
16) JC411	*met* B1, *xyl*-7, *mtl*-2, *mal* A1, *sup* E44, *his*-1, *leu*-6.	CGS # 4274
17) 7019	*aro* B	CGS # 7019
18) DN7-1	*mal* A , *aro* B ; transductant of MND-1 using 7019 as donor selecting Mal^+.	This work
19) 28	produces colicin K	S. Luria (26)
20) A881	produces colicin E3	S. Luria (26)
21) A737	produces colicin E2	S. Luria (26)
22) A779	produces colicin E1	S. Luria (26)
23) CA165	*i* , lac_2, Su_B^+ (λ)	Hirsh (14)*

*This strain was obtained from Dr. Herrington's collection of *E. coli* strains.

Table 1 (Cont'd)

Strain	Genotype[a] and/or Relevant Characteristics	Source
24) CA J64	lac , trp T (λ).	Hirsh (14)*
25) N4316	met, thy, rna	Hirsh (14)*
26) CR63	Su$^+$ serine, F$^+$, λ^S, SupD60.	Bachmann (27)*
27) D10	met , thy, rna	Gesteland (28)*

Note: CGS = Genetic Stock Centre

*These strains were obtained from Dr. Herrington's collection of E. coli strains.

Table 2

List of Bacteriophage Strains and Their Relevant Characteristics

Strain	Gene Affected	Nature of Mutantion	Source
1) L3	e	UAA	J. Owen*
2) JC1926	e	UAA	A. J. Clark*
3) JC1927	e	UGA	A. J. Clark*
4) JC1912	e	UAG	A. J. Clark*
5) J44	e	frame shift (+1)	J. Owen*

*These strains were obtained from Dr. Herrington's collection of phage strains.

donor strain was added to the 20 ml culture of the recipient strain in a 250 ml flask. The mating mixture was incubated at 37°C in a water bath. Samples were drawn at once and at 5 minutes intervals into small tubes with ~1 ml of normal saline, blended for 10 seconds, mixed with 3-4 ml of soft top agar (at 47°C), plated on selective medium (+ NIV mal nal) and incubated at 37°C for 2-3 days.

Transductions: All the transductions were done with the phage P1-cm (24). Phage were prepared in Superbroth (25). The transduction plates were incubated for 4-5 days at 30°C. The transductants were purified and tested further for unselected characters.

Test for Succinate Utilization: The ability to use succinate as carbon and energy source was scored on succinate tryptone plates, the method of Morris (unpublished results). Cells were grown on succinate tryptone agar plates for ~18 hours and then sprayed with 2, 3, 5-triphenyl tetrazolium chloride. Colonies that use succinate turn red. Colonies were also checked on succinate minimal media plates or liquid succinate minimal media overnight at 37°C.

Colicin Sensitivity Tests. For this purpose the method of Plate (26) was followed.

Assay for l-Serine Deaminase: The assay for l-SD was done by the method described previously (29). The results have been presented as μ moles of pyruvate produced by 0.3 ml of a 100 Klett Units (KU at 420 filter) suspension of cells in the whole cell assay in 35 minutes.

Fluoride Resistance: Fluoride resistance was determined by streaking the strain on nutrient agar plates supplemented with 4 mM NaF (sodium fluoride) and incubating at 30°C or 37°C for 24 hours.

Kanamycine and Neomycine Resistance: Kanamycine and neomycine resistance were studied by plating an overnight LB (Luria Broth) culture in soft agar on a rich (LB) medium plates, placing sensitivity discs on the surface and incubating overnight at 37°C. The size of inhibitory area was then measured.

Determination of Growth Yield: Yield of protein per unit carbon provided was determined by a previously described method (30).

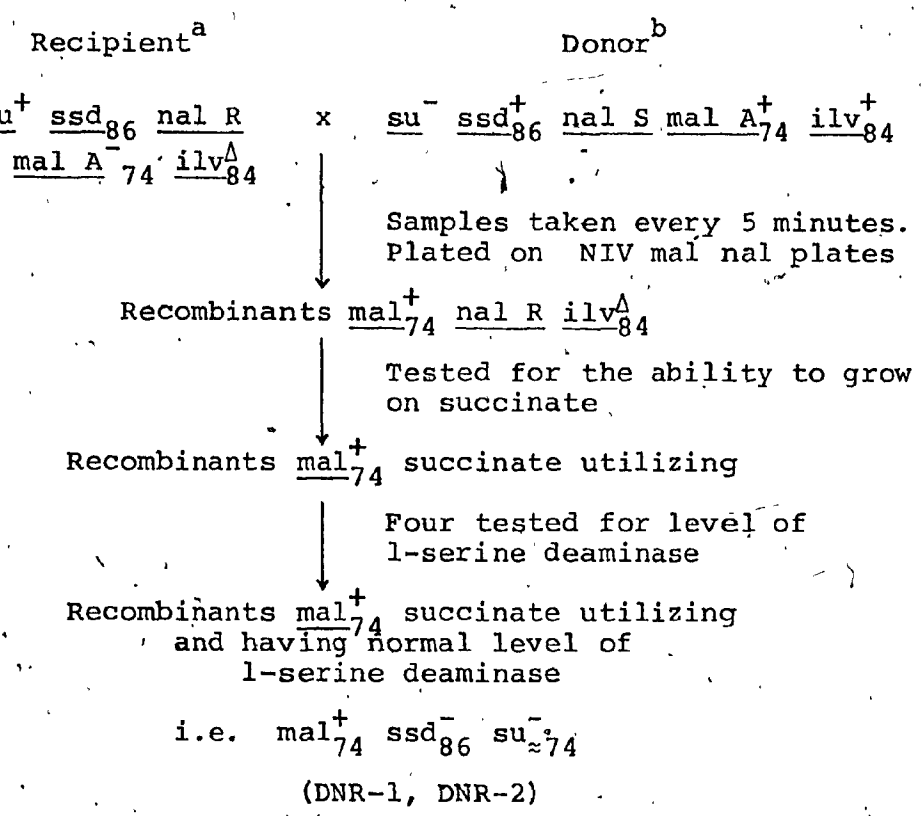
RESULTS

I. Construction of Suppressed Strains Through Interrupted Conjugation

The evidence for the existence of a suppressor of the ssd mutation was found in the following interrupted conjugation experiment. The strain MND-1 which is a nalidixic acid resistant (nal^R), maltose non-utilizing (mal A) derivative of VE2-2, was used as recipient and two Hfr strains, KL14: donating the DNA from 68 minutes clockwise and KL228: donating the DNA from 83 minutes anticlockwise, were used as donors. A selection was made for nalidixic acid resistant and maltose utilizing (nal^R mal A^+) recombinants by plating the mating culture on maltose minimal medium supplemented with nalidixic acid and isoleucine and valine (+NIV mal nal plates). The scheme of the experiment is shown in Figure 1 and the results are presented in Table 3.

Controls made by plating recipient and donor strains on separate +NIV maltose nalidixic acid plates showed no growth because the recipient was mal A^- and the donors were nal^S. Samples taken at 0', 5' and 10' gave no maltose utilizing (mal^+) colonies. The samples taken at 15' were the earliest that gave

Figure 1. Scheme of the conjugation experiment done in order to get suppressed strain.



[a]Donor strains used are - KL14 (PO: 68→69)
KL228 (PO: 84→83)

[b]Recipient strain used is MND-1.

Note: Numericals after the gene symbols represent map location.

Table 3

Recombination Frequencies of Loci mal A_{74} and Suppressor of ssd Mutation

# Cross	Relevant Genotype: Recipient	Donor	Selected Marker	Unselected Marker	Recombination Frequency with Unselected Donor Marker: 0'	5'	10'	15'	20'
1.	MND-1 (ssd^- su^+ nal R ilv^Δ mal A^-)	KL14 (PO: 68→69) (ssd^+ su^- nal S ilv^+ mal A^+)	mal^+ [a]	su^- (ssd) [b]	0	0	0	6/24 (25%)	42/133 (31%)
2.	MND-1 (ssd^- su^+ nal R ilv^Δ mal A^-)	KL228(PO: 84→73) (ssd^+ su^- nal S ilv^+ mal A^+)	mal^+ [a]	su^- (ssd) [b]	0	0	0	40/49 (81%)	102/113 (90%)

[a] mal A^+ scored as ability to use maltose as carbon source.

[b] su^- (ssd) scored as ability to grow on succinate. The level of l-serine deaminase was tested in four succinate utilizing recombinants from each cross and was shown to be low.

maltose utilizing (mal^+) recombinants (24 with KL14 and 49 with KL228 as donor). By 20 minutes the number of mal^+ recombinants increased considerably in both crosses, 133 with KL14 and 113 with KL228: These maltose utilizing (mal A^+) recombinants were tested for their ability to use succinate as carbon source (one of the characteristics affected by the ssd mutation) by growing on succinate minimal medium and also by checking succinate utilization on succinate cryptone plates using tetrazolium spray for test. Out of 157 mal^+ (in cross with KL14) 48 were found to be succinate utilizing (Table 3). In the other cross with KL228 out of 162 mal^+ recombinants 142 were succinate utilizing. Four of these mal^+suc^+ recombinants from each cross were assayed for l-serine deaminase activity (another characteristics of ssd mutation) which was low in all cases (Table 4).

These mal^+suc^+ recombinants (DNR-1, DNR-2) were also tested for the requirement of isoleucine and valine, by growing on minimal medium with and without isoleucine and valine, and were found to require these amino acids for growth and are, therefore, ilv^{Δ}_{84} as was the recipient.

The results of these crosses indicate the existence of a suppressor mutation located in the

Table 4

l-Serine Deaminase Activity in the Mutant, Suppressed and Wild Type Strains

Strain	l-Serine Deaminase Activity[a]	Growth on	
		Maltrose	Succinates
1. MND-1 x KL14 mal^+ suc^+ recombinants (DNR-1)			
#1	7.0	+	+
2	7.4	+	+
3	7.2	+	+
4	7.5	+	+
2. MND-1 x KL228 mal^+ suc^+ recombinants (DNR-2)			
#1	7.6	+	+
2	7.0	+	+
3	7.5	+	+
4	7.4	+	+
3. CU1008 (wild type)	7.9	+	+
4. MND1	>23	-	-

[a] l-serine deaminase was assayed as described previously (29) in cells grown in minimal medium without inducers and is expressed as micro moles of keto acid produced in 35 minutes per 100 K.U. of cells.

region of mal A (since it is transferred with it) and on the mal A side of ilv_{84} (which is not transferred, with it in these crosses). The same suppressor seems to effect both succinate utilization and l-serine deaminase level.

II. Construction of Suppressed Strains Through Transduction

The experiment described in the previous section suggested that there is a suppressor located near mal A (74 minutes). If this suppressor was located within two minutes on either side of mal A it would be possible to transfer the same suppressor by PI cm transduction, since PI cm phage can transfer about two minutes of DNA. That the suppressor is indeed transduced by linkage to mal A is shown in the following section.

The suppressor mutation (su^-) was transduced from strains KL14 and KL228 to the strain carrying the ssd mutation by growing PI cm phage on the donor strains and selecting for maltose utilization ($mal\ A^+$) using a $mal\ A^-$ derivative of VE2-2, MND-1.

The maltose utilizing ($mal\ A^+$) transductants were screened for their ability to use succinate as carbon source. In each transduction, of 600 mal^+ transductants screened only two were able to use succinate (Table 5). These suppressed strains (DNT-1, DNT-2) were assayed for l-serine deaminase activity which was found to be low (Table 6).

Table 5

Co-transduction Frequency of Loci Mal A 74 and Suppressor of ssd Mutation (su^-)

# Transduction	Relevant Genotype: Donor	Recipient	Selected[a] Marker	Unselected[b] Marker	Frequency of Unselected Donor / Marker
1.	KL14 $mal\ A^+\ su^-\ ssd^-$	MND-1 $mal\ A^-\ su^+\ ssd^-$	$mal\ A^+$	su^-(ssd)	2/600 (0.33%)
2.	KL228 $mal\ A^+\ su^-\ ssd^-$	MND-1 $mal\ A^-\ su^+\ ssd^-$	$mal\ A^+$	su^-(ssd)	2/600 (0.33%)

[a] mal^+ scored as ability to grow on maltose minimal medium.

[b] scored as ability to grow on succinate and having low level of l-serine deaminase.

Table 6

l-Serine Deaminase Activity in Suppressed and Mutant Strains

Strain	l-Serine deaminase activity[a]	Growth on Succinate
1) MND-1/KL14 mal^+ suc^+ transductant (DNT-1)	5.8	+
2) MND-1/KL228 mal^+ suc^+ transductant (DNT-2)	5.9	+
3) MND-1/KL228 mal^+ suc^- transductant (DNT-3)	23.8	-

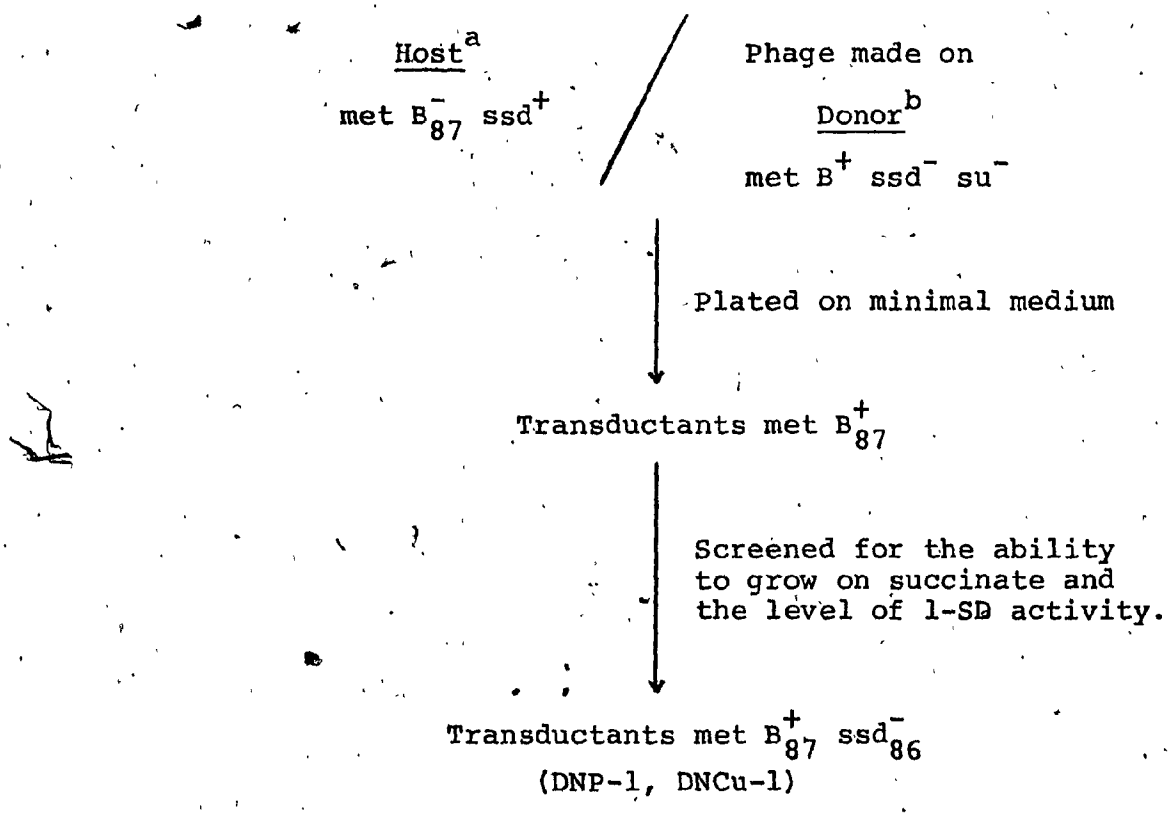
[a] l-SD was assayed as described previously (29) in cells grown in minimal medium without inducers and is expressed as micromoles of keto acid produced in 35 minutes per 100 K.U. of cells.

III. Testing for ssd Mutation in Suppressed Strains

The succinate-utilizing strains described in the preceeding sections were constructed through conjugation and transduction methods and were thought to be suppressed. If these strains are really suppressed, they should still carry the original mutation ssd and one should be able to transduce this mutation to other strains. To determine whether the suppressed strain carries the ssd mutation phage was grown on suppressed strains and used to transduce the ssd mutation to a ssd^+ host. Since the ssd mutation can not be selected directly, a host strain that carried a mutation met B at 86 minutes (which is very close to the ssd locus so that the two loci met B and ssd could be co-transduced) was used (Figure 2) and the selection was made for methionine independence (met B^+). The methionine independent transductants were screened for their ability to utilize succinate (Table 7) and then four of the succinate non-utilizing transductants (DNP-1, DNCu-1) were assayed for the level of l-serine deaminase which was found to be high (Table 8).

These results prove that the succinate utilizing strains described in the preceeding section do carry the ssd mutation and that their ssd^+ phenotype is probably due to the presence of a suppressor of the ssd mutation.

Figure 2. Scheme of transduction of ssd mutation from suppressed strain to a ssd^+ strain.



[a]Host strains used are - P4X met B^- ssd^+
CU1008 met B^-

[b]Donor strains used are - DNR-1 met B^+ ssd^- su^-
DNT-2 met B^+ ssd^- su^-

Note: Numericals after the gene symbols represent map location.

Table 7

Transduction Frequency of Loci met B and ssd

Transduction #	Relevant Genotype Host	Donor	Selected[a] Marker	Unselected[b] Marker	Percent of Unselected Donor Marker
1)	P4X met B^- ssd^+	DNR-1 met B^+ su^- sd^-	met B^+	ssd^-	6/26 (23%)
2)	CU1008 met B^- ssd^-	DNT-2 met B^+ su^- ssd^-	met B^+	ssd^-	20/90 (22%)

[a] met B scored as ability to grow without methionine in the medium.

[b] ssd^- scored as ability to use succinate. The level of l-serine deaminase activity was tested in four succinate non-utilizing transductants from each cross and was shown to be high.

Table 8

Level of l-Serine Deaminase in Strains Transduced with ssd Mutation (Cells were Grown in Glucose Minimal Medium Without Inducer)

Strain	l-Serine Deaminase Activity[a]	Growth on Succinate
1) DNP-1 (met B^+ ssd^-)		
#1	> 23	-
2	> 23	-
3	> 23	-
4	> 23	-
2) DNCu-1 (met B^+ ssd^-)		
#1	> 23	-
2	> 23	-
3	> 23	-
4	> 23	-
3) DNCu-2 (met B^+ ssd^+)	11	+

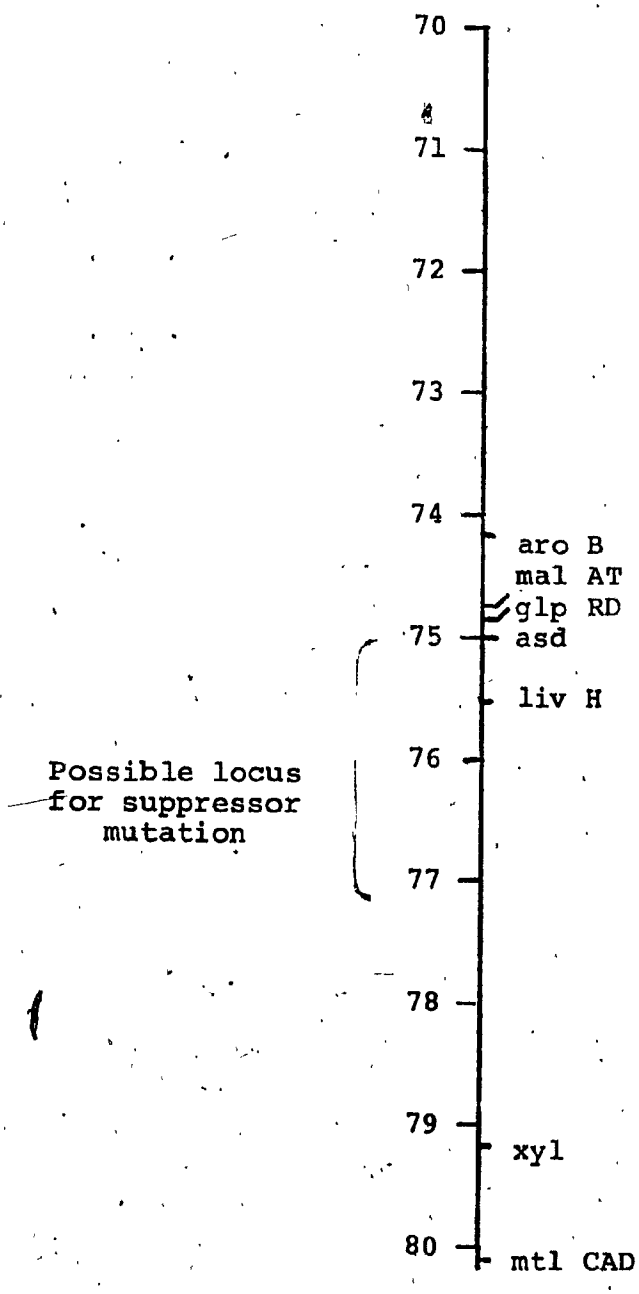
[a] l-SD was assayed as described previously (29) and is expressed as micro moles of keto acid produced in 35 minutes per 100 K.U. of cells.

IV. Closer Mapping of Suppressor Mutation

During the attempts to construct a suppressed strain as described in the preceeding sections a 0.33% (2/600) linkage between the suppressor mutation and the mal A locus at 74 minute was found (Table 5). The suppressor mutation was shown to be linked to the mal A locus but the degree of linkage is very low - so low indeed that an accurate assessment cannot be made. In an attempt to locate the suppressor more accurately, studies were made with some other markers in that area. However, very few markers are known in that region of E.coli linkage map (Figure 3).

Linkage with xyl (80) and mtl (79). The strain 4274 carries mutations affecting xylose and mannitol utilization. These mutations are xyl at 80 minutes and mtl at 79 minutes. A ssd^- derivative of this strain was used as host to study linkage between suppressor (su) and xyl and mtl loci. The phage grown on strain KL14 and KL228 was used as donor. The selection was made for xyl^+ and mtl^+ transductants separately on plates supplemented with xylose and mannitol respectively. Each transduction was carried out three times and 500 transductants were analysed each time. Of 1500 xyl^+ and 1500 mtl^+ transductants, none were able to u[redacted] succinate, thus ruling out the

Figure 3. Linear scale drawing of part of the circular linkage map of E. coli K-12 showing markers of interest.



possibility that the suppressor mutation is within two minutes on either side of xyl_{80} and mtl_{79} locus.

Linkage with $aro\ B_{73}$. To study linkage between suppressor locus and aro B locus, the ssd carrying strain, MND-1, was transduced with the phage grown on strain 7017 which carries the marker aro B at 73 minutes using $mal\ A_{74}$ as selected marker. To this aro B^- strain, DN7-1, suppressor mutation was transduced through the phage grown on KL14 and KL228. Approximately 800 aro B^+ transductants were screened for their ability to use succinate. None of them could use succinate showing that no linkage exists between aro B and the suppressor mutation.

To summarize, the suppressor mutation showed a low linkage to mal A (74 minutes) locus in the transduction experiment. Since the transduction phage carries a maximum of two minutes of the DNA, it could be concluded that there might be a difference of about two minutes between the two loci. Other experiments to map the suppressor through transduction showed no linkage with xyl_{80}, mtl_{79} and $aro\ B_{73}$ loci. An attempt to map the suppressor with two other markers asd (at 73 minutes) and liv H (at 75.5 minutes) is underway. A possible position assessed with the help of these results will be discussed latter.

V. Testing the Nature of Suppressor with Certain Known Informational Suppressors

The suppressor mutation was tested in order to know if it could be identified with a known informational suppressor. The various phages used in this test carry the mutations which could be suppressed by suppressors of UAA, UAG, UGA and frameshift mutations. Only strain KL14 was tested as it is described as a suppressor free strain (27) meaning that it does not carry any of the known informational suppressors. Phages carrying a particular kind of suppressible mutation were grown on a permissive host (which is known to carry the suppressor of that particular kind of mutation), a nonpermissive host (which does not carry the suppressor of that mutation) and strain KL14 (which carries the suppressor of ssd mutation). The number of plaques formed after overnight incubation on rich soft agar medium were counted. The number of plaques on nonpermissive host represent the reversion rate and it much less than the number on permissive host. The results are presented in the Table 9. From the results, it could be concluded that the strain KL14 is not a permissive host of any of the phages and therefore does not carry a suppressor of any of these kind of mutations.

Table 9

Testing the Nature of Suppressor of ssd Mutation with Certain Known Inforamtional Suppressors

Nature of Phage Mutation	Phage Used	Growth on PH[a]	NPH[b]	KL14[c]	Conclusion
1) UAA (ns)	L3	+++	-(2)	-(16)	-ve
2) UGA (ns)	JC1926	+++	-(88)	-(78)	-ve
2) UGA (ns)	JC1927	+++	-(~700)	-(~700)	-ve
3) UAG (ns)	JC1912	+++	-(~10)	-(~20)	-ve
4) Frameshift	J44	+++	-(~10)	-(~20)	-ve

[a]Permissive host: Strain CA165 for UAA, CAJ64 for UGA, CR63 for UAG and N4316 for frameshift mutation.

[b]Nonpermissive host: Strain D10.

[c]Strain to be tested.

(Numbers in bracket represent the number of plaques per plate.)

+++: indicates several thousand plaques per plate.

-: indicates 1-700 plaques per plate.

VI. Comparison of Physiological Characteristics of Suppressed, Mutant and Parent Strains

The various biochemical and physiological characteristics between wild type and a strain with ssd mutation are known to differ. These characteristics, studied in suppressed strains, DNR-1 and DNT-2, include:

1) Growth rate on glucose.
2) Growth rate on succinate.
3) Growth yield on glucose.
4) Growth and adaptation to use other carbon sources; rhamnose and serine.
5) Resistance to fluoride.
6) Resistance to antibiotics; kanamycin and neomycin.
7) Sensitivity to colicins.

Most of these characteristics are shown to be restored by this suppressor mutation as seen in the following description.

Restoration of growth rate on glucose by the suppressor. Strain VE2-2 grows more slowly on glucose minimal medium than does the parent strain CU1008 (2), the apparent doubling of the mutant strain being 80 minutes as compared to 55 minutes for the parent strain CU1008. A comparison of the growth rate of mutant (MND-1), suppressed (DNR-1 and DNT-2) and parent (CU1008) strains shows that the suppressor which restores

growth on succinate also restores the growth rate on glucose to approximately normal (Figure 4). A calculation of apparent doubling time shows that the suppressor does restore the growth rate of the mutant from 90 minutes to ~63 minutes which is almost the same as that of the parent strain which is 65 minutes.

Restoration of growth rate of mutant on succinate by suppressor. The mutant strain MND-1 is unable to grow on succinate and the suppressor restores the ability to use succinate as carbon-source. A comparison of growth rate of C 1008, DNT-2 and DNR-1 on succinate minimal medium was done in order to see if the rate of growth had been restored to normal. The results have been plotted in Figure 5. The apparent doubling time of CU1008, DNR-1 and DNT-2 are 156', 156' and 153' minutes respectively. Therefore, it seems that not only the ability to grow but also the rate of growth on succinate has been restored by the suppressor.

Restoration of growth yield on glucose. The mutant VE2-2 has been shown to use glucose less efficiently than the parent strain C 1008, synthesizing about 15% less cell mass per unit glucose (31). The result of a comparison of growth yield between mutant, suppressed and parent strains, showed that the

Figure 4: Growth curves of mutant (MND-1), suppressed (DNT-2 and DNR-1) and parent (Cul008) strains on glucose (Turbidity of the cells in Klett units vs. time in minutes).

Symbols: (●) for strain Cul008,
(O) for strain MND-1,
(▲) for strain DNT-2 and
(Δ) for strain DNR-1.

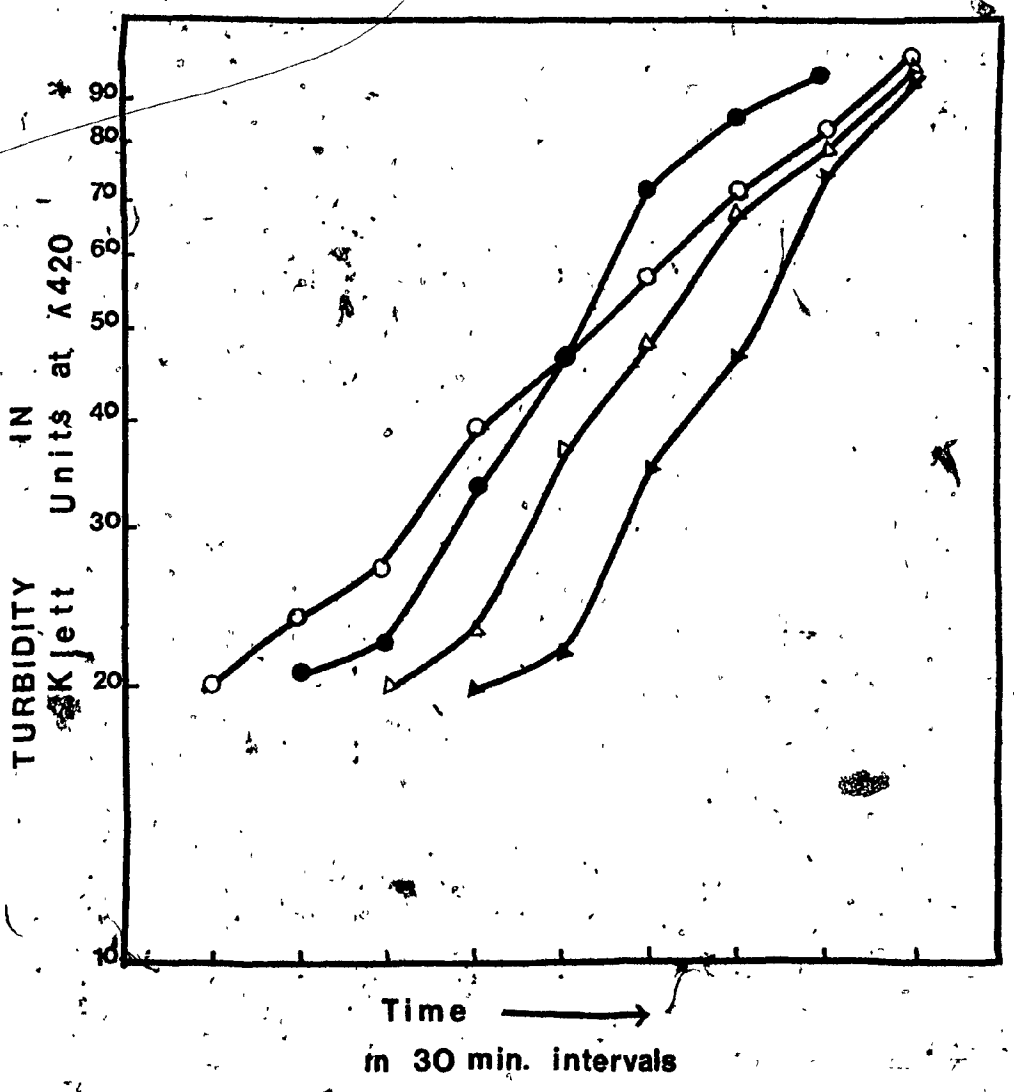

TURBIDITY IN
Jett Units at λ420
90
80
70
60
50
40
30
20
10
Time
in 30 min. intervals

FIGURE 4

Figure 5: Growth curves of mutant (MND-1), suppressed (DNT-2 and DNR-1) and parent (Cu1008) strains on succinate.

Symbols: (●) for strain Cu1008,
(O) for strain MND-1,
(▲) for strain DNT-2 and
(Δ) for strain DNR-1.

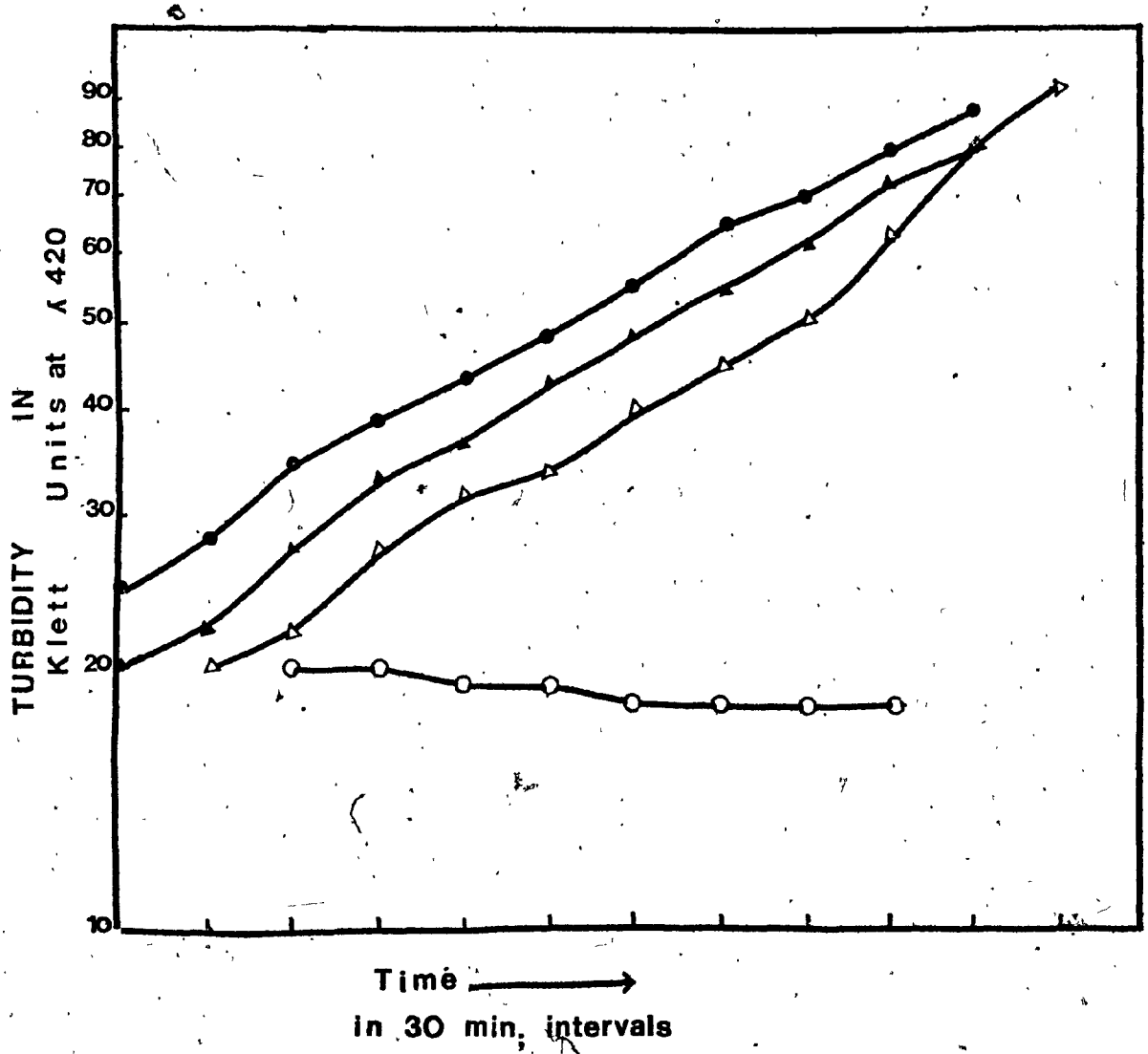

TURBIDITY IN
Klett Units at λ 420
90
80
70
60
50
40
30
20
10
Time
in 30 min. intervals

FIGURE 5

suppressor also restores the efficiency of the mutant to use glucose to normal (Table 10).

Growth and adaptation to other carbon-sources.

Rhamnose: Strains CU1008. MND-1, DNR-1 and DNT-2 were grown on rhamnose minimal medium with and without a limiting amount (0.02%) of glucose, to compare their ability to adapt to growth on rhamnose. The results are given in the Table 11. All the strains grew on rhamnose when supplemented with a little glucose. However, strain CU1008 grows with a shorter lag period when only rhamnose is present in the the medium, while the mutant MND-1 and both the suppressed strains DNR-1 and DNT-2 showed a very long lag period. This finding shows that the deficiency in ability to adapt for metabolizing other carbon source has not been restored by the suppressor.

Serine: Strains CU1008, MND-1, DNR-1 and DNT-2 were streaked on agar plates containing l-serine as sole carbon-source. The parent strain (CU1008) is unable to use l-serine as carbon source while the mutant strain (MND-1) can use l-serine as sole carbon source. If the suppressor returns this character to its parental state, the suppressed strains should be unable to use l-serine. In fact suppressed strains were unable to use l-serine as was the parent.

Table 10

The Effect of Limiting Carbon Source on Yield of Cell Material

Concentration of Glucose	Strains	Final Turbidity in K.Us.	Protein/ml Culture Filterate in µg/ml
1) 0.04%	Cu-1008	125	112
	MND-1	117	91
	DNR-1	140	109
	DNT-2	120	109
2) 0.08%	Cu1008	209	153
	MND-1	192	129
	DNR-1	212	156
	DNT-2	204	154

Table 11

Ability to Use Rhamnose in Mutant, Suppressed and Parent Strains

Strain	Carbon Sources in the Medium		
	Glucose 0.02%	Rhamnose 0.2%	Glucose 0.02% + Rhamnose 0.2%
1) Cu1008	+	++ (12 hrs)	+++
2) MND	+	+ (long lag period, 24 hrs)	+++
3) DNR-1	+	+ (long lag period, 24 hrs)	+++
4) DNT-2	+	+ (long lag period, 24 hrs)	+++

+: indicate turbidity of the culture.

Restoration of resistance to fluoride. Strains CU1008, MND-1, DNR-1 and DNT-2 were streaked on nutrient agar plate with 40 µg/ml of sodium fluoride. The parent strain CU1008 has been found to grow and form colonies at this concentration of fluoride while mutant MND-1 strain does not. Both suppressed strains grew in these conditions. Therefore, the suppressor does restore the ability to grow at this concentration of fluoride.

Resistance to kanamycin and neomycin. Strains CU1008, MND-1, DNR-1 and DNT-2 were grown on rich medium soft agar and a test was done using sensitivity discs of 5 and 30 µgm/ml. The area of inhibition of growth around the discs was measured after incubating overnight at 37°C. The parent was found to be sensitive to the drugs and the mutant was quite resistant (Table 12). The two suppressed strain showed a resistance level in between the parent and mutant, i.e., the suppressor has not completely restored sensitivity to the kanamycin and neomycin.

Resistance to colicins. The mutant, suppressed, and parent strains were tested for their sensitivity to colicins E_1, E_2, E_3 and K. The results indicate that all the four strains have equal sensitivity to colicin E_1, E_2 and E_3 while strain Cu1008 is

Table 12

Sensitivity Discs Test for Neomycin and Kanamycin

Antibiotic	Strain	Width of Annulus of Inhibition Around the Sensitivity Discs (in cms) 5 µgm/ml	30 µgm/ml
1) Kanamycin	Cul008	0.20	0.60
	MND-1	0.00	0.15
	DNR-1	0.15	0.25
	DNT-2	0.00	0.30
2) Neomycin	Cul008	0.30	0.60
	MND-1	0.00	0.20
	DNR-1	0.20	0.40
	DNT-2	0.30	0.50

sensitive to colicin K and the mutant and the suppressed strains are resistant to colicin K (Table 13). This shows that suppressor does not restore the sensitivity to colicin K.

Summary of the Physiological Characteristics Studied

It seems, therefore, that the suppressor which restores growth on succinate and the level of l-serine deaminase activity to normal state restores some of the physiological characteristics but not the others. It completely restores the growth rate and growth yield on glucose, growth rate on succinate, growth on l-serine as sole carbon source and resistance to fluoride. Some of the functions such as sensitivity to the antibiotics kanamycin and neomycin, has been shown to be partially restored by the suppressor while inefficiency in adaptation to grow on carbon source other than glucose, i.e. rhamnose, and resistance to colicin K are the functions that are not influenced by the suppressor.

Table 13

Colicin Sensitivity in Suppressed, Mutant and Parent Strains

Strains	Colicin Senstivity			
	E_1	E_2	E_3	K
1) CU1008	+	++	++	+
2) MND-1	+	++	++	-
3) DNR-1	+	++	++	-
4) DNT-2	+	++	++	-

+ indicates growth

- indicates no growth

DISCUSSION

The work reported in this dissertation provides evidence for the existence and map location, around 75 - 76 minutes, of an extragenic suppressor of the ssd mutation in two strains of E. coli K-12, KL14 and KL228. The biochemical properties of suppressed, mutant and parent strains are compared in order to study the extent to which the various mutant characteristics are suppressed. The ssd suppressor is compared with certain known informational suppressor mutations and appears to work by a different mechanism.

The original or primary mutation ssd (at 86 minutes on E. coli linkage map) has been reported to be pleiotropic affecting many, "apparently" highly diverse characteristics namely, energy metabolism, transport, drug resistance and metabolism of l-serine deaminase (2). The primary target of ssd mutation has not yet been defined (31). Two other mutations, plate's kanamycine resistant mutants (32) and ecf B (26) mutation, which share some of the properties of ssd mutation have been reported. The primary target of these mutations is also not known. During the studies on the ssd mutation, some indication of the presence of an extragenic suppressor was found. It was hoped

that further studies on the suppressor might lead to an understanding of the nature of ssd mutation.

Suppression of the ssd mutation was shown in two interrupted matings between F^- MND-1 (nal^R, mal A, ssd, ilv^Δ) and Hfr KL14 and KL228 (both nal^S). By selecting on plates supplemented with maltose, isoleucine, valine and nalidixic acid, both parents could be counter selected, the F^- because it is maltose non-utilizing and the Hfrs because they are sensitive to nalidixic acid. Among the maltose utilizing (mal^+) recombinants, some were succinate utilizing and had a low level of l-serine deaminase (ssd^+ phenotype). This phenotype could have two possible explanations, first that the mal^+ recombinants have reverted back from ssd^- to ssd^+ and second that the mal^+ recombinants carry suppressor (Su) of the ssd mutation. In the first case the genotype will be ssd^+ and in the second case it will be ssd^- su^-. Since the recombinants giving "ssd^+ phenotype" were able to transduce ssd mutation to another strain, it was concluded that they are suppressed and are of ssd^- su^- genotype.

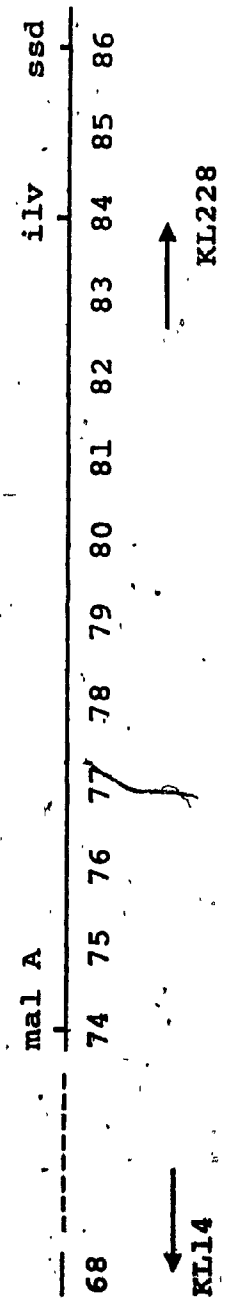
The F^- recipient used in this cross carried an $ilv\ A_{84}$ deletion. Since all the mal^+ recombinants still required isoleucine and valine, they have clearly not received DNA at 84 minutes from the donor. KL14 donates from 68 minutes clockwise and suppresses ssd

without donating ilv A. KL228 donates from 84 minutes anticlockwise and therefore, must be donating a suppressor anticlockwise from ilv A (Figure 6). These observations seem to locate the suppressor approximately between 74 - 84 minutes. The fact that ilv A is not transferred in either cross means that ssd which is at 86 minutes and therefore on the other nontransferred side of ilv A, is also not donated. This confirms that KL14 and KL228 are donating a suppressor and not the functional gene, ssd.

The suppressed strains were also constructed through transduction using same donor and recipient strains. They were also confirmed to have genotype ssd su , by their ability to transduce ssd mutation to another strain P4X.

In the transduction the mal A (74 minutes) locus was used as selected marker and the suppressor was found to be co-transducible with it, though at a very low frequency (2/600). For closer mapping several known markers around the mal A region of the linkage map were used. None of these showed any linkage when transductants were analysed. Unfortunately the region of E. coli chromosome near mal A is not rich in markers which could be easily selected (Figure 3). The marker aro B (at 73.5 minutes) did not show any

Figure 6. Linear drawing of part of circular linkage map of E. coli K-12 showing points of interest.



linkage to the suppressor in the transduction experiments indicating that the suppressor locus is not within two minutes on either side of aro B marker (73.5 minutes) as the transducing phage can carry a maximum of 2 minutes of DNA. Other markers used to map the mutation through transduction were xyl and mtl near 79 and 80 minutes respectively. The suppressor could not be cotransduced with either of these markers. These results indicate that the locus for suppressor mutation is not within two minutes on either side of the distance from xyl (at 79 minutes) and mtl (at 80 minutes). Another marker glp DR in that area was not used since it is very close to mal A and the suppressor showed very low linkage frequency to mal A. Two other marker asd (74.3 minutes) and liv H (75 minutes) were not studied and from the results it was concluded that the region from 75.5 to 76 minutes might be the locus of our interest.

Further work on the mapping of this suppressor of ssd mutation is under way in our laboratory using the liv H mutation as a marker.

The last part of this work deals with the study of the extent to which the various pleiotropic characteristics due to the ssd mutation are altered by the suppressor studied here. The selection for a suppressed strain was based on the restoration of one characteristic only - namely succinate utilization. The suppressed ssd strain grows on succinate, as does the parent. Does it then resemble the parent or the mutant in the other characteristics?

In fact the suppressor that restores succinate utilization also restores some of the other characteristics of the pleiotropic ssd mutation but not all of them. Now a suppressor mutation is itself a single mutation and therefore should result in a single altered gene product. In the present case this can not be the same gene product as was altered in the original ssd mutation because the altered gene product restores some but not all the ssd phenotype.. The mechanism of action of the ssd gene product is not yet clearly understood nor is the interrelationship between the highly diverse phenotypic characteristics affected by this single mutation (31), namely serine and energy metabolism, transport and antibiotic resistance. By considering the nature of those characters which are suppressed and those which are not, perhaps some light

can be shed on the physiological consequences of the ssd mutant itself.

Among the characteristics due to the ssd mutation that are suppressed by the suppressor mutation studied here are the inefficiency in the use of glucose (as judged by the growth rate and growth yield experiments), the ability to use l-serine as sole carbon and nitrogen source, the high level of l-serine deaminase and the sensitivity to fluoride (Table 14). What could be the nature of the single gene product that can alter all these characteristics? This might be clear if we knew the primary factor that relates all these characteristics.

The inefficiency in glucose utilization and the inability to grow on succinate might be due to some change in normal energy metabolism that renders the bacteria unable to use its energy efficiently or to maintain the normal state of energy in the cell. Several such pleiotropic suc⁻ mutants have been reported in E. coli, for example, unc A, unc B, hem A, ubi D, etc. (33, 34, 35, 36) (Table 15).

Another set of characteristics, restored by this suppressor, includes the ability to use l-serine as carbon source and a simultaneously occurring very high level of l-SD. Normally E. coli can not use l-serine as sole carbon source. It seems quite likely that the

Table 14

Physiological Characteristics of $\underline{ssd}^+$ $\underline{su}^+$, $\underline{ssd}^-$ $\underline{su}^+$ and $\underline{ssd}^-$ $\underline{su}^-$ Strains

Characteristics	Genotype of the Strain		
	ssd^+ su^+ (Parent Strain)	ssd^- su^+ (Mutant Strain)	ssd^- su^- (Suppressed Strain)
1. Ability to use succinate	Able	Unable	Able
2. Efficiency of glucose use	Efficient	Inefficient	Efficient
3. Level of l-serine deaminase	Low	High	Low
4. Ability to use l-serine as carbon source	Unable	Able	Unable
5. Sensitivity to colicin produced by strain 28 (colicin k)	Sensitive	Insensitive	Insensitive
6. Sensitivity to fluoride	Sensitive	Insensitive	Sensitive
7. Efficiency of adaptation to carbon source other than glucose	Efficient	Inefficient	Inefficient
8. Sensitivity to neomycin and kanamycin	Sensitive	Insensitive	Partially Sensitive

Table 15

Description of Succinate-Nonutilizing Pleiotropic Mutants

Mutated Gene	Characteristics	Map Position	References
1. unc A, unc B (uncoupled)	Suc^-, Defective ATP-linked transport (glutamine), Normal respiration-linked transport (proline), Neo^R, Kan^R, Col k^R, Normal 1-SD level.	83	33, 34, 35 Newman (unpublished result)
2. hem A (δ-ALA requiring)	Suc^-, Defective respiration-linked transport (proline), Normal ATP-linked transport (glutamine), Neo^R, Kan^R, Col k^R.	26'	35
3. ubi D (ubiquinone)	Suc^-, Defective respiration-linked transport (proline) Normal ATP-linked transport (glutamine), Neo^R, Kan^R, Col k^R.	85'	35

Table 15 (Cont'd)

Mutated Gene	Characteristics	Map Position	References
4. ecf A (energy couping factor)	Suc^{ts}, Defective respiration-linked transport (proline), Normal ATP-linked transport (glutamine), Neo^R, Kan^R, Col k^R.	64'	35, 39, 40, 41.
5. Plates Neo^R mutant	Suc^{ts}, Defective respiration-linked transport (proline), Normal ATP-linked transport (glutamine), Neo^R, Kan^R, Col k^R.	-	32, 35.
6. ecf B (energy coupling factor)	Suc^{ts}, Defective respiration-linked transport (proline), Normal ATP-linked transport (glutamine) Neo^R, Kan^R, Col k^R.	86'	35, 40.

Table 15 (Cont'd)

Mutated Gene	Characteristics	Map Position	References
7. ssd	Suc^-, Defective respiration-linked transport (proline), Neo^R, Kan^R, Col k^R, High l-SD level, Able to use l-serine as sole carbon source.	86'	1, 31.

ssd mutant can use l-serine as sole carbon source because it has a very high level of l-serine deaminase that helps it to break l-serine into pyruvate that could be ultimately utilized as a carbon source. How is this change in carbon metabolism related to the change in energy metabolism? Which one of these two is the primary target of the ssd mutation? What could be the common step between these two metabolic processes that is affected in ssd mutation? These are the questions that still have no definite answer.

The last characteristic of the ssd mutation that has been restored is the sensitivity to fluoride. Since many mutants that have defective carbohydrate metabolism give a low yield on glucose as does the ssd mutant and enolase is the enzyme known to be sensitive to the fluoride an assay was done for the total activity and sensitivity to fluoride by Newman et al. (31) and no alteration was found. Thus the specific locus of fluoride sensitivity in the ssd mutation is not known.

In the light of this analysis of the data, it can only be said that the suppressor restores the carbon (l-serine) and energy related characteristics of the ssd mutation. The nature of the single product that can alter all these characteristics is unknown.

The characteristics due to the ssd mutation that

are not restored to normal are resistance to colicin made by strain 28, inefficiency in adapting to growth on carbon sources other than glucose and resistance to two aminoglycoside antibiotics - namely neomycin and kanamycin. However the resistance to the later two antibiotics has been partially restored by the suppressor. The interrelationship between resistance to antibiotics and the ability to adapt for another carbon source is not obvious at all. Nothing conclusive could be said about the factors involved in the ability of the cells to adapt to an alternate source of carbon. It might have some relation with the altered energy metabolism of the cell since it is known that exhausted cells take much longer to adapt, the exact mechanism involved is not obvious. The question arises as to how the ssd gene product that alters the l-serine carbon and energy metabolism simultaneously brings about the resistance to these antibiotics and why the suppressor is unable to restore the resistance to these antibiotics? This part of the discussion is an attempt to answer these questions in the light of current knowledge about the action and resistance of these antibiotics in bacteria.

The mode of action of colicins, a protein antibiotic, is not clearly understood. However, in

the light of the knowledge available its action could be divided into three stages. At the first stage the colicin molecules are absorbed at the receptor molecules on the membrane through an energy independent process. At the stage no metabolic damage due to colicin occurs. That the molecules remain attached to the surface was evident due to the fact that the cells at this stage can be rescued from the lethal action of colicin by treatment with a protein degrading enzyme, trypsin, that can not penetrate the cell membrane. In the second stage the attached colicin molecules are transferred into the cell through an energy dependent process (37). In the third stage colicin acts on several systems and results in a decrease in intracellular ATP level, inhibition of many transport systems, and arrest of macromolecular synthesis, leading to the death of the cell.

Plate (26) has concluded that the primary target of colicin action is to de-energize the membrane and this then leads to other intracellular colicin triggered destructions. This is based on the fact that an unc A (ATPase defective mutant) mutant which is thought to have a defective membrane energization system, has a decreased sensitivity to colicin K. Since it was the proline transport (depending on respiration-linked membrane energization) that changes drastically before

any other change was observed following the exposure to colicin in unc A mutant, he concluded that the colicin acts primarily on membrane. By de-energizing it, it inhibits the transport of proline which is coupled to membrane energization, before any change in the ATP dependent glutamine transport or in any other system could be seen. Plate et al. (37) have also explained that the difference in sensitivity to colicin between unc^+ and unc A is because in unc^+ cells the de-energized membrane is re-energized by use of ATP (as they have functional ATPase) and this decrease in intracellular ATP leads to further lethal effects of colicin, where as in unc A^- cells due to the absence of active ATPase the ATP is not hydrolyzed and the secondary effects of colicin can not be seen. With this information about the action of colicin various possible mechanisms that a cell can develop to resist colicin can be postulated. A varying degree of resistance could be attained by either inactivating the colicin receptor or by blocking the energy requiring transport of colicin across the membrane, or by changing some site or subsite of colicin action in the cell so that colicin does not bind anymore or by making a colicin degrading enzyme. The unc A mutants show a degree of resistance to the colicin K as a

secondary effect of the unc A mutation. Another set of colicin K resistant mutants studied by Plate et al. (33, 37) showed a pleiotropic phenotype. Since the various phenotypic characteristics were similar to another known peiotropic mutation, ecf A, that has a defect in some unidentified membrane protein invplved in coupling the energy of respiration to the ATP synthesis, they concluded that the colicin resistant mutant also has altered protein that acts as the receptor of colicin as well as has some functional similarities with ecf A they named these mutants as ecf B (38, 39, 40, 41). The ssd mutant is also colicin resistant and has the same pleiotropic nature as the ecf B (Table 15). In addition it also has a drastically changed l-serine metabolism, a characteristic that has not been studied in ecf B mutants. However, it also maps near 86 minutes and Newman et al. (31) think that ssd is at the same locus as ecf B and Plate's mutant. They also suggested that the primary target of the mutations is not the "ecf" membrane protein but is something linked to l-serine and energy metabolism or something entirely different. Now the question arises if the "ecf" membrane protein is not responsible for the resistance to colicin then what is the mechanism of resistance to colicin in ssd mutants? It could be something that is involved in the energy

dependent transport of colicin across the cell membrane, perhaps the state of energy of the membrane itself, or some site of colicin action in the cell. Not much is known about the mechanism of action of colicin in the cell and an enzyme degrading the colicin has not yet been reported. It seems likely that the state of energy of the cell that develops due to the ssd mutation is the cause of colicin resistance. It seems that the cells carrying the ssd mutation have not been making optimum use of energy (as they have low yield on glucose and have a low growth rate too) and perhaps due to this low energy state of the whole cell, they are unable to carry energy dependent transport of colicin K. The fact that a unc A mutant can resist colicin to some extent because of a defect in energy metabolism further supports the view that the ssd mutation resists colicin because of the state of energy of the cell rather than because of some receptor protein in the membrane. Transport studies will give a better understanding of this mechanism because if its the transport of colicin that is affected it will be easy to see if radioactive colicin can be transported into or on the ssd cells or not.

The suppressor mutation is unable to restore the sensitivity to colicin K although it does restore the carbon metabolism and perhaps the energy metabolism

too (as seen by a normal growth rate and growth yield). It could be said that the suppressed strains are able to restore the energy state within the cells but are unable to maintain the energization of the membrane, hence blocking the energy dependent transport of colicin K across the membrane.

The mechanism of action of aminoglycoside antibiotics have been extensively studied and seems to follow the same pattern as does the colicin. The action could be divided into 3 phases (35). The first phase is energy independent, is very rapid and is due to ionic interaction of antibiotics with the cell surface components. This phase does not show any lethal effect of antibiotics. This is followed by the 2nd phase of accumulation of antiobiotic inside cell through an energy dependent processess since it is inhibited by inhibitors of electron transport and oxidative phosphorylation. The 3rd phase is also energy dpendent and needs susceptible ribosomes and corresponds with the onset of the inhibition of protein synthesis and loss of cell viability. Mutants resistant to antibiotics have been found to have either a block in the transport or a non-susceptible ribosome (42). Some strains resist antibiotics by degradative enzymes which are made by the extrachromosomal segment of DNA

(R-factor) (43). None of the genes for various degrading enzymes have been found to exist on the E. coli chromosome itself. The factor responsible for transport of drug across the membrane (2nd phase) is not yet known. It is known that this stage of drug accumulation needs energy from electron transport because hem$^-$ mutants defective in $\bar{e}$ transport chain have high resistance to drugs associated with decreased accumulation. When given the substance that restores the electron transport chain, these mutants develop an increased sensitivity and accumulation of the drug. Since the uncoupling agents that do not block electron transport can also decrease the entry of drug, it seems obvious that electron transport is not acting directly as a carrier system but only as an energy source.

As the ATPase$^-$, unc A$^-$, mutants have high sensitivity and increased accumulation of drugs the carrier system clearly does not need an active ATPase of membrane. Indeed, loss of activity causes increase in the entry. ATPase$^-$ mutants with proton hole in the membrane also have decreased accumulation and therefore increased resistance to the drug. Even colicin k resistant mutants (ecf B$^-$) have a decrease in accumulation and an increase in resistance to the drugs. Carrier for various sugars and other compounds structurally

related to the drugs are not involved in antibiotic transport since, no competition was seen when transport studies were done using drugs and the other compounds. Since accumulation was inhibited by some divalent ions it was suggested by Bryan et al. (35) that probably streptomycin is transported through a carrier that is involved in transport of some ions. Another interesting fact about the transport of drug is that only the aerobic energy is utilized for the transport since anaerobic bacteria are insensitive to these drugs as they are unable to transport the drug across the membrane. Certainly the ssd mutant has an altered energy system that is involved in transport of these drugs but what is that single factor changing the whole energy system is not clear. The suppressor seems to restore the carbon and energy systems inside the cell to a normal state, but it only partially brings back the sensitivity to the antibiotics. Why it is so is not clear. Perhaps some drug is still transported through that state of membrane energy or may be drug accumulation depends on the energy state of both, the membrane and the inside of the cell. Since the suppressor is bringing only the intracellular environment to normal, the sensitivity is only partially restored.

The uncertainty about the nature of the primary

target of ssd mutation still remains. It does not seem to be a carrier protein in the membrane, as suggested by Plate et al. (33, 37) because it is very difficult to even speculate a situation where an altered protein in the membrane can alter drastically the l-serine metabolism in the cell. One can suggest that the ssd gene product changes the energy state of the inside of the cell as well as of its membrane and the suppressor restores the intracellular state of energy to normal but is unable to do the same for the membrane. However, it is hard at present to express this in mechanistic terms. In any case it seems that the ssd gene product plays a very important role in energy status of the cell.

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