STUDIES ON A MECHANISM PROVIDING FOR GENETIC TRANSFER IN *SERRATIA MARCESCENS*[1]

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The demonstration of genetic transfer in bacteria is dependent not only upon finding the proper experimental conditions which permit genetic transfer to occur, but also upon finding adequate screening methods for identifying the new genotypes. In those few cases where genetic transfer has been demonstrated, a variety of mechanisms have been described. Transformation of genetic characters by the treatment of viable cells with highly purified deoxyribonucleic acid extracts derived from closely related strains has been demonstrated in the *Pneumococci* (Avery, McLeod and McCarty, 1944; Austrian, 1952), in *Hemophilus influenzae* (Alexander and Leidy, 1951) and in *Escherichia coli* (Boivin, 1947). Transduction, in which genetic materials are transferred by bacteriophages, has been demonstrated between several *Salmonella* species (Zinder and Lederberg, 1952) and in *E. coli* (Morse, 1954). Bacterial recombination has been described in *E. coli* (Lederberg and Tatum, 1946), *Achromobacter fischerii* (McElroy and Friedman, 1951) and *Shigella* species (Luria, 1955).

There is no *a priori* reason to assume that the ability to exchange or transfer genetic materials is limited to these few bacterial species. In fact, it seems likely that if the proper experimental conditions were known, genetic transfer would be demonstrable in many, if not all, bacterial species. This suggestion receives support from the evidence presented in this paper for genetic transfer in *Serratia marcescens*.

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MATERIAL AND METHODS

Organisms. S. marcescens is a fast-growing, pigmented organism which has proved its usefulness in previous genetic studies (Bunting, 1946). Since it is closely related to the enteric group, in which three of the known mechanisms for genetic transfer are represented, it seemed quite possible that some form of transfer could be detected. Since *S. marcescens* grows well on simple defined media, and mutations leading to specific biochemical requirements can be induced with relative ease (Hemmerly, 1953), conventional methods for discovering genetic transfer would be appropriate.

The particular strains used in this work were biochemically deficient mutants (auxotrophs) derived from the HY strain of *S. marcescens* by ultraviolet irradiation. The origin, symbols, and requirements of the auxotrophic strains are shown in figure 1. These strains were maintained in stock culture by serial transfer on peptone-glycerol-phosphate slants.

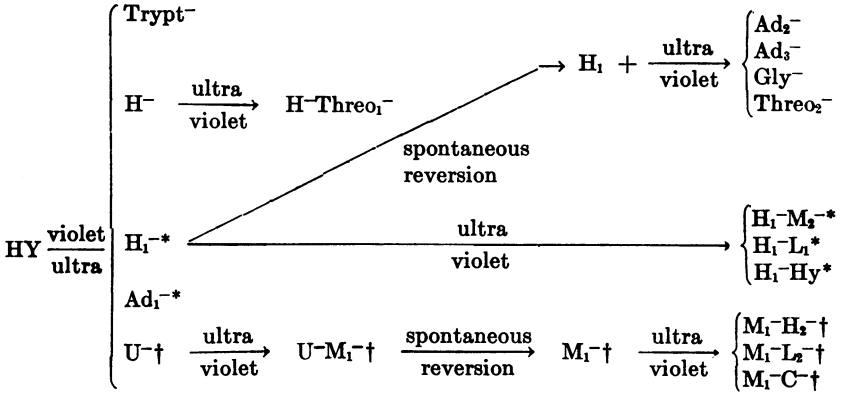
Media. The defined medium used in this work was similar to that used in previous studies (Labrum and Bunting, 1953) and will be referred to as S medium. Its composition was: K_2HPO_4 (anhydrous), 8.0 g; ammonium citrate, 5.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $Fe(NO_3)_3 \cdot 9H_2O$, 0.02 g; glycerol, 20 ml; and distilled water, 1,000 ml. For the isolation of auxotrophic mutants, 2.0 g peptone (Difco) were added per L of S to give S + P medium.

The peptone-glycerol-phosphate medium which was used for stock transfers was composed of: peptone, 5.0 g; K_2HPO_4 (anhydrous), 2.0 g; glycerol, 10 ml; 1,000 ml distilled water, plus 2 per cent agar.

Beef heart infusion broth (BHI) was used for growing inoculum cultures for all experiments.

Mutant isolation. S. marcescens produces large amounts of penicillinase which makes the penicillin method (Lederberg and Zinder, 1948, Adelberg and Myers, 1953) of mutant isolation

Figure 1. Auxotrophic strains and their derivation symbols, and requirements



Symbol	*Requirement*
Ad⁻	Adenine
C⁻	Cysteine
Gly⁻	Glycine
H⁻	Histidine
Hy⁻	Hypoxanthine
L⁻	Leucine
M⁻	Methionine
Threo⁻	Threonine
Trypt⁻	Tryptophan
U⁻	Uracil

* These strains act as donor strains, and are comparable to the H_1 strain. They comprise Group I.

† These strains act as acceptor strains, and are comparable to the M_1 strain. They comprise Group II.

impractical. However, when an irradiated suspension of *S. marcescens* was plated on S + P agar, and small colonies were picked after 5 days, the progeny of about one-third of these colonies proved to be auxotrophic. This gave a reasonably efficient method of obtaining auxotrophs for further study.

Drug resistant strains were isolated by the gradient plate technique (Szybalski, 1951).

Reverse mutation from nutritional dependence to independence was measured by the method described by Lieb (1951). The rate for mutation $H_1^- \rightarrow H_1^+$ was 6.93×10^{-10} per bacterium per division cycle and for $M_1^- \rightarrow M_1^+$ was less than 6×10^{-9} per bacterium per division cycle.

EXPERIMENTAL RESULTS

Prototroph formation in mixed cultures. Preliminary screening of a number of auxotrophic strains in pure and mixed cultures in liquid medium suggested that some sort of interaction between strains did occur. Two of these auxotrophic strains H_1^- and $U^-M_1^-$ grew to visible turbidity when mixed in liquid S medium, while neither of the two strains gave evidence of growth in pure cultures. When the turbid mixture was streaked on S agar plates to discriminate between prototroph formation and cross feeding, prototrophs, whether the result of genetic exchange or back mutation, were found to be present.

In order to determine whether there was a higher rate of prototroph formation per cell in the mixture than in the single parents, the two auxotrophs H_1^- and $U^-M_1^-$ were plated in pure and mixed suspensions on S agar plates. Since the medium did not support the growth of either deficient parent, it was selective for prototrophs. Experiments of this type were set up according to the following standard procedure: Duplicate 10-ml BHI broth tubes were inoculated with the auxotrophic strains and incubated 18–24 hr at room temperature with shaking. An equal volume of BHI broth was added at the end of this time, and incubation continued for 1–3 hr to ensure log phase cultures. These cultures were then washed twice in 0.85 per cent saline by centrifugation and decantation, and the pellets finally resuspended in

TABLE 1

Pooled data from 29 crosses, showing the mean frequency of prototroph formation in single and mixed cultures, with statistical analysis of the data

	Prototrophs Arising in Individual Parental Suspensions		Prototrophs Arising in Mixture
	H_1^-	$U^-M_1^-$	Both
Mean #/10^8	0.115	0.147	7.11
Variance	0.051	0.063	43.43
Variance of mean	0.0017	0.0022	1.4975
Std. error of mean	0.042	0.046	1.224

Degrees of Freedom = 28; $P < 0.001$ by T test.

liquid synthetic medium. Equal volumes of the two parental suspensions were mixed for the cross and allowed to stand for 15–20 min at room temperature. The mixture, and each parental suspension separately, were plated on S plates (0.1-ml aliquots of undiluted suspension). In most cases 10 plates were inoculated with the cross, and 5 plates each with the parental suspensions. The parental suspensions were also plated at the appropriate dilutions for titer assay on enriched medium. All plates were incubated at 29 C. The assay plates were counted at 48 hr, and reversion controls and cross plates were scored at 5 days.

The results of 29 crosses done over an 18-month period have been pooled to yield the data in table 1. The number of prototrophs occurring in the mixed culture was significantly and reproducibly higher than the number appearing in either parental suspension. The possibility was considered that reversion had been somehow accelerated in mixed populations, and was responsible for the increased number of prototrophs found in such cultures. Several experiments devised to test this possibility gave negative results, and the data to be described in later sections yielded positive evidence for actual transfer. Among the tests which indicated an absence of accelerated reversion or nonspecific mutagenic action were: (1) Crosses of both the H_1^- and $U^-M_1^-$ strains with F^+ and F^- strains of *E. coli* strain K-12, which gave negative results; (2) comparison with minimally enriched controls, which simulated the conditions which would occur if syntrophy took place (this did not result in any increase in prototrophs); (3) mixtures of H_1^- and $U^-M_1^-$, both of which are sensitive to streptomycin, failed to produce an increase in the number of streptomycin resistant cells over the individual parental controls, when plated on SM plates. In addition, only certain mixtures gave increased numbers of prototrophs (i. e., H_1^- x M_1^-, $H_1^-Hy^-$ x M_1^-, Ad_1^- x M_1^-), while others (H_1^- x $M_1^-L_2^-$ and Ad_1^- x H_1^-) did not; this fact suggested a more specific mechanism than reversion or random increase in mutation frequency.

In order to determine whether prototroph formation in mixed culture was peculiar to the H_1^- and $U^-M_1^-$ strains, a number of crosses were carried out using strains with dependence upon other biochemical compounds. Among these mutant strains were Ad 1, Ad 2, Ad 3, Gly, and Threo 2, requiring adenine, glycine, and threonine respectively. Each of these strains was crossed with both H_1^- and $U^-M_1^-$, and all of them were capable of prototroph formation when mixed with one or the other of the original strains. These results are in accord with the idea of a rather general ability of strains to participate in prototroph formation and indicate that this ability is not restricted to the H_1^- and $U^-M_1^-$ strains. Furthermore, the fact that there was some specificity among the strains with regard to their ability to cross with one *or* the other of the two original strains was further evidence that reversion was not responsible for prototroph formation. The strains could be grouped into two groups with regard to their ability to cross with either H_1^- or $U^-M_1^-$, giving indication of some sort of polarity (figure 1). Strains which yielded prototrophs when mixed with $U^-M_1^-$ could be placed along with the H_1^- strain in group 1, and the other strains would comprise group 2, and would include the $U^-M_1^-$ strain. This was the first evidence of a fertility difference, and later evidence from crosses involving the H_1^- and $U^-M_1^-$ strains was consistent with these findings.

Experimental variations such as length of preincubation of the mixture prior to plating, the effect of temperature at 25, 29, and 37 C, and population density, as well as the ratio of the parents to each other, were found to have no significant effect upon the frequency of prototroph formation.

Characteristics of the Mechanism. Filtration and U-tube experiments. Of primary interest was the question of filtrate activity, since two of the known mechanisms for genetic transfer in bacteria are associated with factors that are separable

from whole cells by filtration. In transduction this factor has been closely identified with bacteriophages (Zinder and Lederberg, 1952) and in transformation the cell-free factor has been identified as deoxyribonucleic acid (Avery, McLeod, and McCarty, 1944). In the third mechanism, bacterial recombination, cellular contact is required for genetic transfer.

Filtrates were prepared from various strains at different times, and tested by mixing with viable strains. In no case were filtrates capable of promoting prototroph formation; control crosses with the cultures from which the filtrates were derived were fertile.

Although these results suggested that cell contact might be required for prototroph formation to occur, it could be argued that the agent, once freed from the cell, was labile under the conditions of the experiment, or that it was necessary (as it was in transduction experiments with Salmonella) for a carrier to pass from one cell to a cell of the complementary auxotrophic type, and then back to the first. In an effort to approximate more closely the conditions of a cross without actually permitting contact, and so test these arguments, a modification of the U-tube (Davis, 1950) was devised.

The two auxotrophic strains H_1^- and $U^-M_1^-$ were inoculated into liquid minimal medium, one on each side of the sintered glass filter. The medium was alternately washed back and forth through the filter by the application of gentle negative pressure on each side of the filter, thus permitting optimum exposure of each strain to the filterable materials from the other, without permitting actual contact between intact cells to occur. Aliquots from both sides of the filter were plated at various time intervals after inoculation. The results are tabulated in table 2. The control tubes in table 2 consist of each parental strain inoculated separately into liquid S medium as a reversion control, and the column labeled "mixture" represents a control cross between the two strains to yield prototrophs in this experiment. The numbers of organisms in all tubes were in the same order of magnitude, and no visible increase in turbidity was obtained up to 48 hr of incubation. No prototrophs were formed as a result of constant exposure of the strains to the filterable materials from each other, nor in the reversion controls where each parent was incubated separately. In the control cross, however, prototrophs were observed in spite of the relatively small numbers of parental cells.

TABLE 2

Numbers of prototrophs arising in liquid minimal medium in pure and mixed culture, and in the modified U-tube

Time of Sampling	Control Tubes:		From U-tube:		Mixture*
	H_1^-	$U^-M_1^-$	H_1^-	$U^-M_1^-$	
Zero.....	0	0	—	—	—
40 min...	0	0	0	0	1
2 hr.....	0	0	0	0	2
9.5 hr....	0	0	0	0	1
48 hr.....	0	0	0	0	50

* These are the actual numbers of prototrophs obtained from 0.2 ml of the liquid minimal medium from the tubes. The titer was approximately 5×10^7 by visual estimation in all tubes at the time of inoculation, and at the termination of the experiment. No visible increase in turbidity occurred during the 48 hr of incubation.

No samples were taken from these tubes at time zero; identical suspensions were used to prepare all tubes.

These results support the previous observation on lack of activity of filtrates, and indeed, the failure to obtain prototrophs in either case strongly suggests that cell contact is required before prototrophs can be formed. Since genetic transfer is mediated by an agent separable from whole cells by filtration in both transformation and transduction, the *S. marcescens* transfer would seem to resemble *E. coli* recombination more closely.

Stimulating effect of ultraviolet light. The stimulating effect of ultraviolet light upon the frequency of genetic exchange in bacteria has been observed by a number of investigators (Hayes, 1952; Zinder and Lederberg, 1952). In the transduction system, prototroph formation is increased as a result of interaction of cells with increased numbers of phage particles liberated from cells by ultraviolet irradiation, but in bacterial recombination no adequate explanation has been advanced for the stimulatory effect.

In common with the above systems, ultraviolet irradiation has been found to have a profound effect upon prototroph formation in *S. marcescens*. Cultures of the two auxotrophs H_1^- and $U^-M_1^-$ were grown and washed by centrifugation and decantation. The washed organisms were resuspended in liquid minimal medium and aliquots of

TABLE 3

The stimulating effect of ultraviolet irradiation of one parental strain prior to mixing upon prototroph formation in mixed culture

Ultraviolet Dose in Sec		Surviving Number of Cells		% Survival		Reversions/Ml Surviving Fraction		Prototrophs/ Ml Mixture
H_1^-	$U^-M_1^-$	H_1^-	$U^-M_1^-$	H_1^-	$U^-M_1^-$	H_1^-	$U^-M_1^-$	
None	None	1.1×10^9	1.8×10^9	100	100	0	4	18
30″	None	9.5×10^8	1.8×10^9	88	100	16	4	290
60″	None	4.0×10^8	1.8×10^9	37	100	18	4	780
90″	None	5.0×10^7	1.8×10^9	5	100	16	4	1,150
120″	None	5.0×10^6	1.8×10^9	0.5	100	33	4	2,550
None	30″	1.1×10^9	6.4×10^8	100	35	0	0	77
None	60″	1.1×10^9	5.0×10^8	100	27	0	4	102
None	90″	1.1×10^9	8.0×10^7	100	4.5	0	2	120
None	120″	1.1×10^9	8.0×10^6	100	0.45	0	0	92

each parental suspension were irradiated. Samples were withdrawn from the irradiation mixtures at various time intervals and the treated and untreated suspensions diluted with BHI broth. The tubes were stored for 1 hr in the dark, then washed, resuspended, and assayed. Crosses were set up by mixing equal volumes of the suspensions, and all crosses and parental suspensions were plated for prototroph estimation. The results are given in table 3. The differences in per cent survival in various cultures given identical doses of ultraviolet irradiation are not necessarily a function of differential resistance to ultraviolet, but are probably a result of different initial population densities or volume of the treated sample, since no effort was made at standardization along these lines.

In this experiment, the effect of ultraviolet irradiation was most pronounced on the histidine dependent strain, giving greater than a 100-fold increase in prototroph numbers with 120″ exposure, which killed 99.5 per cent of the cells. (The dose was 3 erg/mm²/sec at a distance of 7 in.) A smaller but significant increase in the number of prototrophs was obtained when the uracil-methionine dependent strain was treated, but the number of prototrophs did not increase beyond 65 per cent kill with this strain.

Experiments were never run using ultraviolet sterilized cultures of either parent, but this might prove to be a productive line of approach. Treatment of either strain with streptomycin, however, resulted in complete sterility, and even streptomycin inactivated cultures containing as many as 10^5 viable cells had completely lost the ability to produce prototrophs. Sonic lysates, even those containing 10^3 viable cells, were incapable of prototroph formation.

The stimulatory effect of ultraviolet light upon prototroph formation facilitated further experimentation since it insured recovery of a reasonable number of prototrophs from crosses involving the H_1^- and $U^-M_1^-$ strains. Other strains in group 1 (figure 1) were stimulated to the same extent as was the H_1^- strain, while those in group 2 behaved similarly to the $U^-M_1^-$ strain, so the phenomenon was not limited to the H_1^- and $U^-M_1^-$ strains alone.

The fact that the absolute numbers of prototrophs increased while the number of viable cells was markedly decreased could be interpreted as evidence for unidirectional transfer, since the ultraviolet light could be considered to "activate" certain cells to donate genetic material to others upon contact, and it would not be necessary for these cells to be viable in order to make this donation, but the cells receiving the genetic material would necessarily have to be viable in order to express the information carried by that material. The fact that irradiation of either the H_1^- or $U^-M_1^-$ strain resulted in an increase in prototroph numbers, suggested that each population contained both donor and acceptor cells. The greater stimulation of the H_1^- strain by ultraviolet light might result from a larger proportion of donor cells in that population, while the small effect with the $U^-M_1^-$ strain would imply that this population contains at least a small proportion of cells capable of acting as donors. This point will be discussed further in the light of other experimental evidence.

Possession of this technique for recovery of

larger numbers of prototrophs permitted a more critical analysis of the requirement for cell contact as a necessary condition for prototroph formation. Although previous attempts to demonstrate a filterable agent capable of inducing prototroph formation were negative, it seemed likely that under these conditions of increased prototroph formation it might be possible to observe an effect which had previously escaped detection because of infrequent occurrence. It was possible that treatment with ultraviolet light might be liberating bacteriophage or some other material from the treated cells in a manner similar to that shown by Lwoff *et al.* (1950) with *Bacillus megaterium*, and that these particles might be responsible for the increased numbers of prototrophs. Since phages have been demonstrated in *S. marcescens* (Wassermann and Seligman, 1953; Adams and Wade, 1954), it seemed advisable to test this possibility.

A histidine-requiring strain was treated with ultraviolet light, as previously described, and the treated suspension divided into two portions. One portion was filtered through an ultrafine sintered glass filter, and the filtrate mixed with an equal volume of a cell suspension of the $U^-M_1^-$ cells. The other portion, containing the treated H_1^- cells, was mixed with a cell suspension of the $U^-M_1^-$ strain directly. The results support the original observation that cell contact is required for prototroph formation; no prototrophs were formed when the filtrate was mixed with the $U^-M_1^-$ cells, while in the cross, where cell contact occurred, 324 prototrophs per ml were obtained in one experiment, and 210 prototrophs per ml in the second. Therefore, there was no evidence that filterable units could effect genetic transfer, even though the irradiation experiments had indicated that it was not necessary that the donor be a viable cell. Obviously, negative results can suggest, but never prove, the absence of a particle. It was conceivable that there could be small active units which for some reason were held back or inactivated by the filter. If such were responsible for exchange, they were not susceptible to trypsin or deoxyribonuclease which were added to the H_1^- strain during the 1-hr dark recovery period following irradiation. Neither enzyme, separately or in combination, had any significant effect upon prototroph formation when treated cell suspensions were mixed with M_1^- cells in the usual manner, as shown in table 4. The lack of evidence for active filterable units indicates that genetic transfer in *S. marcescens* requires participation of whole cells, as does recombination in the K-12 strain of *E. coli*.

TABLE 4

Effect of treatment of ultraviolet irradiated strains with DNAase and trypsin

H_1^- Strain	Enzyme Treatment	Mean Number of Reversions /Ml	Cells/Ml Surviving	Mean Number of Prototrophs/Ml When Mixed with $U^-M_1^-$ Strain
Viable....	None	0	2.2×10^8	2
120″ u.v..	DNAase	2	9.8×10^7	244
120″ u.v..	Trypsin	4	7.3×10^7	182
120″ u.v..	Trypsin DNAase	10	5.5×10^7	374
120″ u.v..	None	6	8.1×10^7	324

Tests for recombination. Whereas transduction involves transfer of apparently small segments of the genetic material, in recombination a transfer and exchange of larger portions of material (and of linked genetic determinants) appears to be involved. A crucial test for the latter phenomenon utilizes the recombination of nonselective markers which occurs when, presumably due to crossing over, prototrophs are formed from strains carrying different biochemical requirements to which the nonselective markers are linked. In order to find out whether recombination did actually occur in *S. marcescens*, the H_1^- and $U^-M_1^-$ strains were marked with resistance to one or more of the following compounds: Streptomycin Sulfate (SM), Chloramphenicol (CM), and Sodium Azide (Az). The resistant strains were selected by the gradient plate method (Szybalski, 1951).

Crosses were set up between these drug resistant strains and plated on S agar plates. Thus, by selecting for nutritional independence and studying the drug resistance patterns of the prototrophs, one might expect to recover recombination types characterized by being doubly resistant, or sensitive, in addition to the parental drug resistant types. The results of some of these crosses involving drug resistant strains are shown in table 5. Out of 150 prototrophic colonies tested in these experiments, no recombination types were observed. In addition, the inconsistency among prototrophs derived from identical crosses with regard to the frequency of occurrence of the parental drug resistance types was

TABLE 5

Distribution of nonselective markers in prototrophs from crosses involving the drug resistance characters CM^r and SM^r

Cross Number	Drug Resistance of Auxotrophs		Reversions per 10^8		Prototrophs per 10^8	Per cent of Prototrophs Resistant to:				Number of Prototrophs Tested
	H_1^-	$U^-M_1^-$	H_1^-	$U^-M_1^-$	Mixture	SM	CM	CMSM	Sens.	
1	—	CM	0.00	0.10	64.6	—	100	—	—	20
2	—	SM	0.00	0.10	9.1	65	—	—	35	20
3	SM	—	0.00	0.10	6.0	35	—	—	65	20
4	SM	CM	0.00	0.10	7.4	55	45	—	—	20
5	SM	CM	0.30	0.03	6.6	31	69	—	—	16
6	SM	CM*	0.00	0.00	156	100	—	—	—	42
7	SM	CM	1.00	0.15	5.3	5.4	94.6	—	—	36
8	CM	SM	0.07	0.00	12.2	—	100	—	—	20
9	CM	SM	0.07	0.00	14.5	—	100	—	—	20
10	CM	SM	0.07	0.00	13.8	—	100	—	—	20

* Reverted to CM sensitivity.

quite obvious. Neither of these two observations was characteristic of a recombination type system, where consistency from cross to cross should be obtained, and recombination between nonselective markers should occur with measurable frequency.

Before drawing any conclusions from these data, it was important to discover the reason for the lack of reproducibility in drug resistance patterns of the prototrophs. One possibility was that variation in the proportions of donor and acceptor cells in the parent strains was responsible. Replicate crosses made with aliquots from the same suspensions did indeed give far more consistent results than those made at the same time with suspensions from different isolations. More convincing evidence was obtained, however, in an experiment where ultraviolet light was used. Proceeding on the assumption that ultraviolet irradiation could effectively convert a heterogeneous population into a donor population, and that this conversion would cause a predictable shift in the drug resistance patterns of the prototrophs, a series of crosses was set up in which one or the other parent was irradiated. A control cross between untreated cultures was also set up. Prototrophs were isolated at random from each cross, and tested for their drug resistance. All crosses were between the two strains H_1^-/CM^rSM^r and $U^-M_1^-/Az^r$. The results of this experiment are shown in table 6. Although the number of prototrophs tested in cross I was small, it was evident that the majority (75 per cent) carried the Az resistance marker of the $U^-M_1^-$ strain, while the remainder (25 per cent) carried the CM^rSM^r of the H_1^- strain. These results could arise if, assuming that drug resistance was not simultaneously transferred with the biochemical marker, 75 per cent of the prototrophs were formed by H_1^- (donor) to $U^-M_1^-$ (acceptor) transfer, and 25 per cent by $U^-M_1^-$ (donor) to H_1^- (acceptor) transfer. Irradiation of the H_1^-/CM^rSM^r population prior to mixing (cross II) should then effectively reduce the number of H_1^-/CM^rSM^r cells which could act as acceptors and permit only the first type of transfer. As the results indicate, prototrophs from this cross were 100 per cent Az resistant, as would be expected on this hypothesis. In cross III, irradiation of the $U^-M_1^-$ strain gave the predicted shift in the other direction, to 85 per cent CM^rSM^r prototrophs, and 15 per cent Az resistants. Further irradiation of the $U^-M_1^-$ population might be expected to give 100 per cent CM^rSM^r prototrophs, but this has not yet been tested.

The results in cross I could have resulted from linkage between the Az and H^+ loci, with simultaneous transfer of both from $U^-M_1^-$ donor cells to H_1^- acceptor cells, but failure to obtain the recombination class $CM^rSM^rAz^r$ and the results of crosses II and III suggest that the Az^r locus was not linked to any of the selective (biochemical) markers studied.

It was concluded that the inconsistencies in drug resistance patterns shown in table 6 were

TABLE 6

The effect of ultraviolet irradiation on drug segregation ratios when one or the other parent was irradiated prior to crossing

	Ultraviolet Dose		Number Surviving		Reversions per Ml		Protos per Ml	No. Protos Tested	Per cent Resistant to*	
	H_1^-/CMSM	$U^-M_1^-$/Az	H_1^-/CMSM	$U^-M_1^-$/Az	H_1^-/CMSM	$U^-M_1^-$/Az	Mixture		CMSM	Az
I	None	None	1.3×10^9	1.5×10^9	0	2	8	8	25	75
II	120″	None	3.8×10^8	1.5×10^9	24	2	1,490	50	0	100
III	None	120″	1.3×10^9	5.0×10^8	0	4	74	38	85	15

* Only parental drug types were recovered among the prototrophs tested. No prototrophs resistant to CM, SM, CM Az, or SM Az were observed.

probably the result of different proportions of donor and acceptor cells in the parent populations. On this assumption, failure to recover recombinant types could best be explained by assuming that in *S. marcescens* only a small portion of the genetic complement was transferred from the donor to the acceptor cell in a given exchange. Thus the selective medium may have permitted the recovery of prototrophs resulting from exchange of units responsible for the chemical syntheses, but the nonselective drug markers may have remained associated with the genotype of the acceptor cell and not have been involved in the genetic exchange.

Crosses with multiple biochemical markers. In view of the failure of drug markers to recombine under nonselective conditions, it was of interest to determine whether recombination could occur with other markers. To test this, the H_1^- and $U^-M_1^-$ strains were irradiated and mutants displaying additional biochemical deficiencies were isolated. Crosses were set up between these multiple auxotrophs, and the markers were studied selectively by plating on synthetic medium, and nonselectively in various combinations by plating on singly and doubly supplemented synthetic media. Several interesting facts came to light as a result of these experiments. Most significant of these was that the addition of a second biochemical deficiency to the H_1^- (donor) strain did not affect the rate of prototroph formation (table 7), while addition of a second biochemical requirement (other than uracil) to the M_1^- (acceptor) strain completely abolished prototroph formation. The results of several crosses appear in table 7. These data support the hypothesis that only small units were transferred at each exchange, and that the H_1^- strain was predominantly responsible for donating and the M^- strain for accepting the unit exchanged. In no case was there any prototroph formation when both parental strains carried multiple markers, which would necessitate the simultaneous transfer of more than one unit. Thus it was evident that even though whole cell participation was required for genetic transfer to occur in *S. marcescens* there was no evidence of transfer of large units of genetic material, making true recombination possible.

TABLE 7

Effect of addition of a second biochemical deficiency on the formation of prototrophs

Cross	No. Prototrophs/Ml.
H_1^- x M_1^-	174
$H_1^-Hy^-$ x M_1^-	190
$H_1^-L_1^-$ x M_1^-	130
H_1^- x $M_1^-C^-$	0
$H_1^-Hy^-$ x $M_1^-C^-$	0
$H_1^-L_1^-$ x $M_1^-C^-$	0
Ad_1^- x M_1^-	1,050
Ad_1^- x $M_1^-L_2^-$	0
Ad_1^- x $M_1^-C^-$	0
Ad_1^- x M_1^-	1,660
Ad_1^- x $M_1^-H_2^-$	0

In each cross, the first strain was treated with ultraviolet light prior to mixing, to increase the prototroph yield.

Somewhat of a biochemical anomaly became apparent when crosses between double auxotrophs were plated on supplemented synthetic medium; only unit transfer from the donor (H) to the acceptor (M) strain was observed. In table 8, the results of one such cross between $H_1^-Hy^-$ and $M_1^-L_2^-$ are presented. Considering the number of colonies appearing on plates supplemented with methionine alone, methionine plus histidine, and methionine plus hypoxanthine, great disparities were observed. When the colo-

TABLE 8

Prototrophs per 0.1 ml. in excess of reversions, appearing on supplemented minimal media plates from the cross of $H_1^-Hy \times M_1^-L_2^-$

Ultraviolet Dose		Per cent Survivors		Number of colonies/0.1 ml. when cross is plated on synthetic medium supplemented as follows:*								
$H_1^-Hy^-$	$M_1^-L_2^-$	$H_1^-Hy^-$	$M_1^-L_2^-$	None	H	Hy	M	L	H+M	Hy+M	H+L	Hy+L
None	None	100	100	0	0	0	56	0	116	65	0	0
60″	None	10	100	0	0	0	752	0	1,564	984	0	0
120″	None	1.1	100	0	0	0	264	0	430	252	0	0

* Key to supplements: H = Histidine; Hy = Hypoxanthine; L = Leucine; M = Methionine.

nies were tested, all of them were satisfied by methionine as the sole supplement. None of the cells were doubly deficient. This anomaly may have its basis in some sort of sparing action by histidine or hypoxanthine on the methionine requirement. Growth curves of M^- cells in limiting methionine with and without additions of histidine or hypoxanthine were run, and the results support this latter suggestion.

DISCUSSION

Evidence has been presented which indicates that genetic transfer does occur in *S. marcescens* strain HY, and that it presents a combination of features quite different from those characterizing recombination, transduction, or transformation.

All attempts to effect exchange with filtrates have been unsucessful. Only when whole cells were brought into direct contact did transfer occur. In this respect the system resembled that known in *E. coli*.

Irradiation of parental cells with ultraviolet light increased the frequency of subsequent recovery of cells with genetic transfers in spite of the loss of viability involved. The effect was always more pronounced with one parental strain than the other. Since it was apparent that only viable cells could reveal the results of genetic transfer, the differential effects suggested a donor-acceptor system in which the effect of irradiation was primarily to stimulate the donor strain, and could be used to detect a donor strain, or kill off any acceptor cells in a heterogeneous strain. However, the data were not sufficiently clear cut to indicate conclusively whether nonviable cells could participate as donors in transfer, whether the stimulating effects of ultraviolet irradiation did indeed depend upon its lethal action, or whether it had any stimulating effects on acceptor cells. In any event, the irradiation effects were similar to those previously described for *E. coli*.

When crosses were made with auxotrophic strains carrying multiple biochemical deficiencies, the results indicated that only small units of genetic material were transferred. Except in the case of uracil and methionine, which may be very closely linked, no transfer was ever observed which involved more than transfer of a single locus or a single determinant for a hereditary characteristic. The results with multiple deficient auxotrophs provided further evidence for the donor-acceptor system, since it was found that the presence of multiple deficiencies on the donor cells did not interfere with prototroph formation, whereas multiple deficiencies in the acceptor strain effectively prevented it.

Crosses made with auxotrophic strains carrying nonselective drug markers provided additional evidence that genetic transfer in *S. marcescens* did not involve simultaneous transfer of multiple loci, and that a donor-acceptor system was operating. When crosses were made with non-irradiated cells, the prototrophs carried either parental drug marker, in proportions which varied from cross to cross. However, when one parent was irradiated, which inactivated the acceptor cells in that population, the resulting prototrophs carried the nonselective marker of the untreated strain, indicating that the transfer of genetic material had been from the cells of the irradiated strain to those of the viable untreated strain. Again, there was no evidence of recombination of multiple factors.

Thus, the mechanism of genetic transfer in *S. marcescens* resembles the *E. coli* recombination system in requiring whole cell participation, but is more like the transduction system in that only small units of genetic material are involved in each exchange.

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SUMMARY

Evidence has been presented for a mechanism of genetic transfer in *Serratia marcescens* which appears to combine certain features of bacterial recombination and the transduction-transformation type of systems. The characteristics of this mechanism are as follows: (1) Contact between cells is required for genetic transfer to occur. (2) A fertility system, characterized by a donor-acceptor relationship among the mutants, appears to be involved. (3) The frequency of prototroph formation is enhanced by irradiation with ultraviolet light. Differential stimulatory effects of ultraviolet on different parent strains were noted and are believed to reflect the heterogeneity of the populations in regard to donor and acceptor cells. (4) The genetic determinants are apparently transferred on a unitary basis, i. e., only a small portion of the donor cell's genetic material is transferred to the acceptor cell, resulting in unequal contributions of the two parental cells to the prototroph progeny. This conclusion is supported by the fact that addition of a second biochemical requirement to a methionine-requiring strain, which has been identified as an acceptor strain, prevented prototroph formation, while the addition of a second biochemical marker to a histidine-requiring donor strain was without effect on prototroph formation. (5) The donor-acceptor relationship does not appear to be identical with that determining the F^+-F^- relationship in *E. coli*. This was indicated by the failure to recover prototrophs following treatment of donor populations with streptomycin.

ADDENDUM

During the preparation of this manuscript, a communication was received from Dr. B. S. Holloway (1955) in Australia, describing similar work carried out by him in *Pseudomonas aeruginosa*. There were several aspects of this work suggestive of the mechanism found in *Serratia marcescens*, although the similarity is not complete.

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