GENETIC EXCHANGE IN SALMONELLA[1]

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Genetic investigations with many different bacteria have revealed parallelisms and some contrasts with the biology of higher forms. The successful application of selective enrichment techniques to the study of gene recombination in *Escherichia coli* (Tatum and Lederberg, 1947; Lederberg *et al.*, 1951) suggested that a similar approach should be applied to other bacteria. This paper presents the results of such experiments with *Salmonella typhimurium* and other *Salmonella* serotypes. The mechanism of genetic exchange found in these experiments differs from sexual recombination in *E. coli* in many respects so as to warrant a new descriptive term, transduction.

MATERIALS AND METHODS

Most of the strains of *S. typhimurium* were provided by Lilleengen (1948) as representative of his 21 "phage types", LT-1 through LT-22. Most if not all strains of *S. typhimurium* are lysogenic (Boyd, 1950), and these have provided 12 lines of bacteriophage. Other cultures were obtained from F. Kauffmann, E. K. Borman, and P. R. Edwards. All cultures were maintained on nutrient agar slants.

Specific growth factor dependent mutants (auxotrophs) were obtained from ultraviolet irradiated cell suspensions subjected to the penicillin method for selective isolation (Davis, 1950*a*; Lederberg and Zinder, 1948). Similar mutants have been obtained in *Salmonella* by Plough *et al.* (1951) and Bacon *et al.* (1951). Other methods for the isolation and characterization of auxotrophic and fermentation mutants have been documented elsewhere (Lederberg, 1950; Lederberg and Lederberg, 1952). Streptomycin resistant mutants were selected by plating dense, unirradiated cell suspensions into agar containing 500 mg per L of dihydrostreptomycin.

"Complete" indicator medium (EMB) was made up from the same formula as for *E. coli* (Lederberg, 1950). The defined eosin methylene blue medium ("EML agar") contained in g per L: sodium lactate, 2.5; $(NH_4)_2SO_4$, 5; NaCl, 1; $MgSO_4$, 1; K_2HPO_4, 2; methylene blue hydrochloride, 0.05; eosin Y, 0.3; and agar, 15. Difco products, penassay broth, and nutrient agar, were employed as "complete" media.

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Unless otherwise stated, all cultures were incubated at 37 C, and plates were scored after 24 and 48 hours.

EXPERIMENTAL RESULTS

Direct crosses: platings of mixed cultures. In *E. coli*, recombinants were detected selectively by plating various auxotrophs together on minimal agar. Both parents are suppressed on this medium and, barring various experimental errors, colony formation is confined to prototrophic recombinant cells. Such errors can be detected by appropriate controls but are best mitigated by the use of double

TABLE 1

Mutant strains and symbols used

NO.	MUTATIONS	PERTINENT SYMBOL
LT-2	Type 2 prototrophic	Prot
SW-272	Methionineless, auxotrophic	Aux
SW-414 (LA-2)	SW-272 histidineless	
LT-22	Type 22 parent	
SW-240	Phenylalanine and tyrosineless	
SW-279 (LA-22)	SW-240 tryptophanless	
SW-307 (LA-22)	SW-279 galactose-negative	Gal−
SW-351 (LA-22)	SW-307 xylose-negative	Xyl−
SW-435 (LA-22)	SW-351 streptomycin-resistant	S^r
SW-479 (LA-22)	SW-435 mannitol-negative	Mtl−
SW-443 (LA-22)	SW-435 maltose-negative	Mal−
LT-7	Type 7 parent	
SW-184	Prolineless	
SW-188	Methionineless	
SW-191	Leucineless	
SW-481	SW-184 galactose-negative	
SW-492	SW-188 galactose-negative	
SW-503	SW-191 galactose-negative	
SW-514	LT-7 streptomycin-resistant	
SW-515	SW-503 streptomycin-resistant	

nutritional mutants (diauxotrophs). These are obtained by the iterated isolation of mutants in previously established auxotroph lines.

One of Lilleengen's strains was refractory to our techniques of mutant isolation. Two-step mutants with mutually complementary nutritional requirements were prepared from each of the remaining twenty types. Of the two hundred possible pairwise combinations, including "selfed" crosses, one hundred were tested. Each combination was studied by mixing and plating 10^9 washed cells of the two parents on a minimal agar plate. Fifteen mixture plates and five control plates for each parent by itself were inoculated in each test. Fifteen combinations yielded prototrophs in contrast to barren controls. Strain LA-22 was the most "fertile", especially with LA-2 (see table 1). This cross yielded

about one prototroph per hundred thousand parental cells plated. Crosses in which LA-22 was not involved gave prototrophs so infrequently and sporadically as to be of doubtful significance. It has since become evident that LA-22 is genetically a single, stable mutant although it was derived in two steps and has a complex nutrition.

LT-22 is lysogenic for a virus (hereafter referred to as PLT-22) active on LT-2. This virus is capable of inducing lysogenicity in LT-2. Among the lysogenic derivatives of LA-2 three different interaction groups were found: the majority no longer interacted with LA-22 to give prototrophs; a few interacted with impaired efficiency; still fewer were not affected in this respect. These experiments indicated that genetic exchanges did occur and that latent bacteriophage played some role in the interaction.

Indirect crosses: platings of cells and filtrates. To test the possible role of filtrable factors in this interaction, a U-tube with an "ultra-fine" sintered pyrex filter partition was prepared according to Davis's (1950*b*) design. By alternating suction between the arms of the tube, two intact populations of growing bacteria could be made to share the same medium. The integrity of the filter was verified in control experiments by leaving one compartment uninoculated. Then 10^8 cells of each parent were inoculated into twenty ml of broth and placed in either arm of the tube. Ten ml were flushed from side to side every twenty minutes for four hours while the culture grew to saturation. The two populations were washed and plated upon minimal medium. Prototrophs appeared in the platings of LA-22 but not of LA-2. Sterile filtrates of LA-2 broth cultures did not elicit prototrophs from LA-22. However, filtrates from mixed cultures of LA-2 and LA-22 elicited about one prototroph per million LA-22 cells. Thus LA-2 produced a filtrable agent (*FA*), under stimulation from LA-22, that could elicit prototrophs from LA-22. Filtrates of LA-22 cultures, containing substantial amounts of phage (PLT-22) active on LA-2, also stimulated *FA* production from LA-2. The role of this phage will be discussed later.

To help the further exposition of our experiments, we shall use the term transduction for genetically unilateral transfer in contrast to the union of equivalent elements in fertilization. The working hypothesis that *Salmonella FA* is an agent of genetic transduction provides a useful frame of reference for our discussion.

Assay of FA. Stock *FA* was prepared by growing LA-22 and LA-2 in mixed culture in broth. After 48 hours, the cells were sedimented and the supernatant passed through a sintered pyrex filter. The sterility of a filtrate was verified by inoculating samples into broth at the time of preparation and by platings in agar as controls for particular experiments. This precaution was taken although complete sterility is not critical to most experiments since more than a million cells of LA-2 per plate are needed to interact with LA-22 to give prototrophs in the "direct crosses". These preparations have been stored in the refrigerator for several months without loss of activity.

A standard procedure for assay of *FA* was developed for further work. LA-22 was grown on nutrient agar plates and harvested in dense saline suspension.

The viable count was obtained by plating suitable dilutions on nutrient agar. Various dilutions of cells were plated with a constant volume of an *FA* preparation on minimal agar. Prototrophs appeared at 24 hours and were counted after 48 hours. Figure 1 shows that a constant response was found with about 10^9 to 10^{10} cells per plate. The decline at high cell densities was probably due to overcrowding and inhibition of colony formation, and at lower densities to physical separation of cells and agent or to the saturation of susceptible cells.

10^9 cells of LA-22 were plated with serial dilutions of *FA*. Over a considerable range a linear relationship was found between the yield of prototrophs and

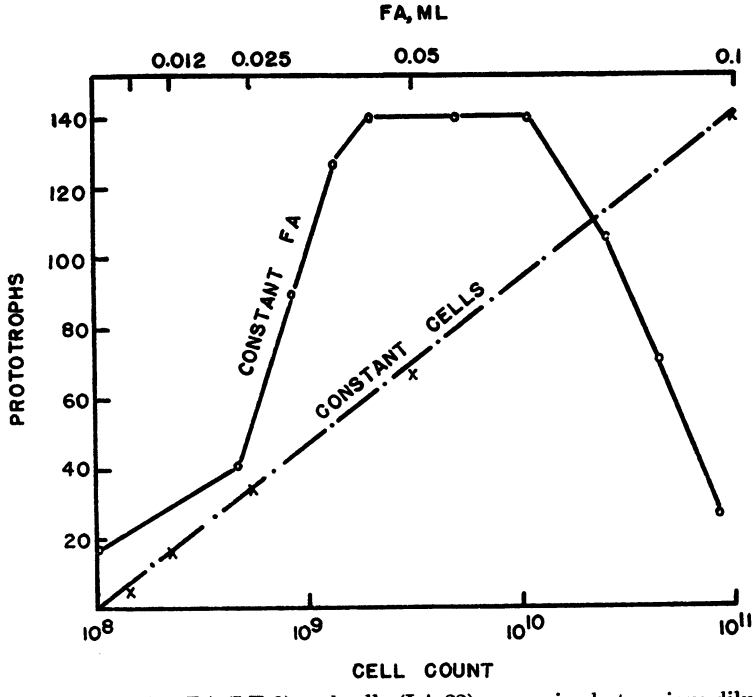


Figure 1. Assay of *FA*. *FA* (LT-2) and cells (LA-22) were mixed at various dilutions and plated on minimal agar. Prototrophs were counted after 48 hours.

amount of *FA* (figure 1). The effect of higher concentrations of *FA* will be discussed in a later section.

A unit of *FA* may be defined as the content of a filtrate that will elicit a single prototroph from an optimum concentration of LA-22 cells. Filtrates from mixed cell preparations usually contain about 2,500 such units per ml.

Chemical reactivity of FA. With the development of a standardized assay it was possible to compare the effects of various treatments on *FA* and bacterial cells. The latter are sterilized by shaking with such agents as chloroform, toluene, alcohol, and formalin. Of these only formalin inactivates *FA*. The bacteria are sterilized by heating at 56 C for 30 minutes. Temperatures of 70 C are necessary for detectable effects on *FA*. It is rapidly inactivated only when 100 C is approached.

FA is quantitatively precipitated from broth by one to two volumes of cold alcohol or half saturated ammonium sulfate. A heavy floc appears in both cases which, for the most part, remains water insoluble; *FA*, however, redisperses.

None of several enzymes tested affected *FA*. They were added directly to the active filtrates and incubated for two hours. The tests included pancreatin (100 mg/ml), trypsin (100 μg/ml), Taka-diastase (100 mg/ml), ribonuclease (10 μg/ml), and desoxyribonuclease (20 μg/ml). The failure of desoxyribonuclease to inactivate *FA* was of particular interest. Enzymatic activity was verified by testing samples of the reaction mixture for reduction of the viscosity of thymus nucleic acid (kindly supplied by Dr. R. D. Hotchkiss). Similar controls were not done for the other enzymes.

Evocation of FA. The properties of phages latent in *Salmonella* have been summarized by Burnet and McKie (1929) and Boyd (1951). Lysogenic phages, i.e., those obtained from lysogenic bacteria, poorly lyse sensitive cultures and readily provoke secondary resistant lysogenic forms. Visible lysis of sensitive cells is observed only at low multiplicities of infection. With higher multiplicities there is little evidence of lysis. When phage is added to broth cultures, the tube does not clear, and the bacteria grow at a reduced but significant rate. PLT-22 is typical of these phages.

To determine whether PLT-22 was unique in its *FA* evoking activity, a variety of treatments was applied to resting and growing cells of LT-2 strains. *FA* was not detected in the filtrates of young cultures or after autolysis with benzene, extraction of dried cells, treatment with high concentrations of antibiotics (penicillin, bacitracin, and aureomycin), or complete phage lysis. Dilute antibiotics, lithium chloride, and crystal violet yielded variable *FA*. High activity is most readily detected in the filtrates of cultures treated with lysogenic phages. These results indicate that *FA* is not released by mechanical, chemical, or biological disruption of cells. However, various deleterious agents elicit its appearance in a way that may parallel the action of latent phage. The most effective concentrations of these reagents were those which only slightly inhibited the cells. *FA* has also been detected in aged cultures when autolysis sets in. This may be due to the action of mutant lysogenic phage.

The production of *FA* in response to chemical stimuli has not yet been sufficiently controlled to give consistent yields needed for experimental use. However, when a filtrate containing little or no *FA* is prepared after treatment of LA-2 with such agents as crystal violet or penicillin and reinoculated into broth with LA-2, *FA* is released in large amounts. This procedure has been carried through for five cycles. The apparent regeneration of *FA* was probably due to a lysogenic phage which had been released in the first treatment. The lack of a reliable indicator for this phage has hindered the analysis of this reaction. However, it has been a useful tool for the evocation of *FA* from a single strain without the introduction of extraneous bacteria or viruses.

Morphological and physical studies. FA has been quantitatively sedimented and recovered in the Spinco ultracentrifuge at 100,000 G for 30 min. Partial sedimentation occurred in the International centrifuge with multispeed head at 20,000 G. In these preparations *FA* is, therefore, of more than macromolecular

dimensions. Filtration through a series of gradocol membranes (obtained through the courtesy of Dr. S. E. Luria) was also used to estimate the particle size. Ten to twenty per cent of *FA* were retained by a membrane of A.P.D. 420 mμ, seventy per cent at 230 and 170 mμ, and ninety-nine per cent at 120 mμ. These results indicate a particle size slightly less than 0.1 μ (Bawden, 1950).

FA preparations exhibit numbers of small, barely resolvable, granules under the phase contrast microscope. Electron micrographs show granules whose size is in rough agreement with the estimates of *FA* from filtration experiments (figure 2). Some of the granules agglutinated with anti-O serum. Visible floccules which can be removed by centrifugation appeared in the reaction tube. However, the activity remained intact in the supernatant. Upon incubation with antiserum, some of the granules enlarge and by four hours have attained sizes of

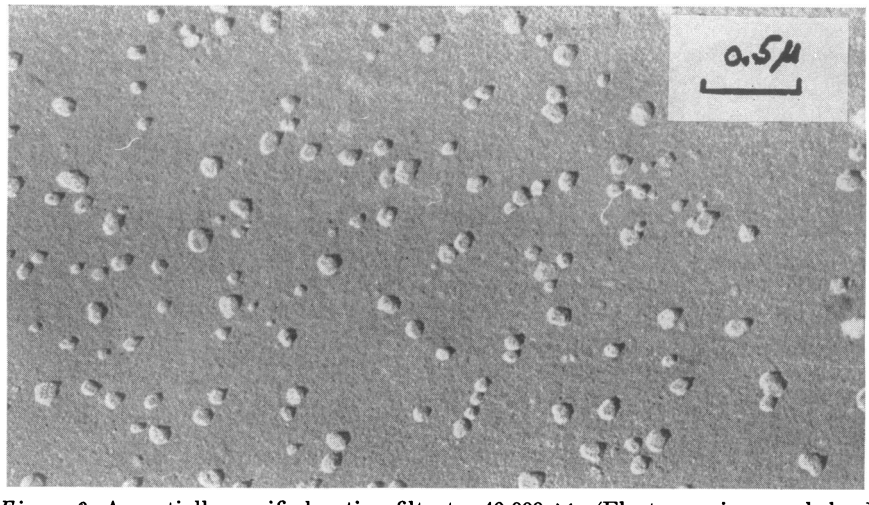


Figure 2. A partially purified active filtrate, 40,000 ×. (Electron micrograph by Dr. Paul Kaesberg.)

5 to 8 μ (see figure 11, Lederberg *et al.*, 1951). These "large bodies" formed mixed floccules with added bacterial cells.

Treatment of *Salmonella* with *FA*-eliciting phage or penicillin results in the formation of chains after one and one-half hours of incubation, and by three hours only "snakes" with swollen bulbular central portions are present (Fleming *et al.*, 1950). Debris and small granules are also seen. *FA* has also been produced by this time. The supernatants of these cultures were difficult to sterilize by conventional means. Filtration through eight or fourteen pound test Mandler candles resulted in filtrates with a viable count of about 100 per ml. Comparable filtrates of untreated cultures have regularly proven to be sterile. Sintered pyrex "UF" filters were found to be suitable for sterile filtration of active filtrates.

These observations are reminiscent of the L-forms of bacteria particularly as interpreted by Klieneberger-Nobel (1951), Dienes and Weinberger (1951), and

Tulasne (1951). There is, however, no evidence of a functional relationship between L-forms and transduction. We have not yet succeeded in obtaining L-colony growth from our cultures that would permit more direct tests, nor have other workers made genetic analyses of L-type growth to fortify speculations on their role in a life cycle.

Sources and range of activity of FA. FA has been defined thus far as a specific product of strain LA-2 with the single capacity of transducing a particular mutant of LT-22. However, other direct crosses involving LA-22 had given prototrophs. To determine if *FA* could be obtained from other strains, a simplified test was applied, involving the selection of streptomycin resistant prototrophs, "SRP" (Lederberg, 1951*a*). SW-435 (LA-22 S^r) was grown in mixed culture with each of fifty different wild type (streptomycin sensitive prototrophs) *S. typhimurium* strains, and the mixture plated on minimal agar containing 500 mg per L of streptomycin. Twenty-eight of the crosses yielded evident recombinants, showing that *FA* could probably be produced by many strains.

FA has been isolated from each of twenty-five tested strains of *S. typhimurium* when the proper stimulus was found. PLT-22 served for the many strains susceptible to it, which probably explains the success of the SRP crosses, while other lysogenic phages (from the Lilleengen series) stimulated other strains resistant to PLT-22. In general, inoculation of 10^9 cells of *S. typhimurium* and 10^8 to 10^9 particles of a lysogenic phage to which it is susceptible into 10 ml of fresh broth will yield *FA* after four hours of incubation. Penicillin in low concentrations (one to five units per ml) was successful for some cultures.

A demonstration of recombination in *Salmonella* was initially sought and found in terms of the recovery of prototrophs from mixed platings of auxotrophs. A more complete proof of typical sexuality would depend upon the occurrence of new combinations of "unselected markers" (Lederberg, 1947). SW-478 (LA-22 Gal−, Xyl−, Mtl−, S^r) was crossed with SW-414 (LA-2 Gal+, Xyl+, MTl+, S^s) on EML agar containing one of the various sugars so that one unselected fermentative character could be scored directly on the cross plate. Of some 20,000 prototrophs screened, none differed from SW-478 except in their nutrition. In addition to mutational differences, LA-22 and LA-2 differed intrinsically in ability to utilize malate, alanine, or succinate as the sole carbon source required for growth. All of the prototrophs resembled LA-22. With a total of eight unselected markers there was no evidence of co-segregation. These experiments were repeated with active filtrates from LT-2 and gave the same result.

Genetic transfers for each of three markers (one nutritional and two fermentative) were observed when experiments were set up in such a way as to select for them. SW-435 (Aux, Gal−, Xyl−, S^r) was plated with *FA* (from LT-2 Prot, Gal+, Xyl+, S^s) on minimal, EMB galactose and EMB xylose agar. Upon the EMB media there first appeared a thin film of growth (pink and hence nonfermenting) and then small outcroppings or papillae which fermented the galactose or xylose. These papillae grow quite large (figures 3 and 4) because of their utilization of the sugar when other nutrients are depleted. The xylose-negative mutant gave some papillae due to spontaneous reversion

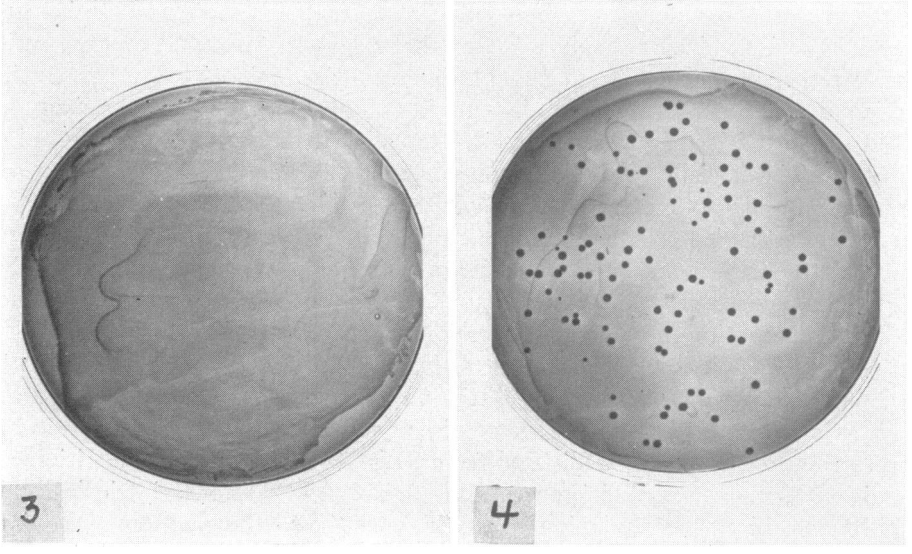
but not enough to interfere with the scoring of the test. The galactose negative mutant is more stable and has only rarely reverted. The number of papillae on EMB or prototrophs on minimal agar (table 2) was approximately the same so that the efficiency of transduction for different selected characters may be uniform. However, the unselected markers remained unaltered; that is, all prototroph selections were nonfermenters and the papillae selections acted only upon the

TABLE 2

SW-435 and FA upon different selective media

MEDIUM	*FA*	BOILED *FA* (CONTROL)
Minimal	120 prototrophs	0 colonies
EMB galactose	114 papillae	0 papillae; film of bacteria
EMB xylose	138 papillae	15 papillae; film of bacteria

Figures are colonies or papillae per plate.



Figures 3 and 4. SW-435 plated on EMB galactose agar with heat inactivated (3) and active (4) *FA*.

one sugar and were auxotrophic. All of the transduced cells were still streptomycin resistant.

The foregoing experiment was repeated on double sugar agar. Individual papillae fermented either galactose or xylose and were all auxotrophic. Because of a slight difference in texture it was possible to differentiate the two kinds of papillae directly on the indicator plate. Entire papillae were picked and transferred to the alternative sugar and to minimal agar. Among the many tested, no mixed papillae were found. Any such could be detected by this rigid selection.

From these experiments, we conclude that an *FA* filtrate has many activities, producing many different transductions (but no more than one per cell) that result in singly transduced clones.

We have observed no linked segregations such as had been found in *E. coli* recombination. The singular activity of *FA* might still be reconcilable with a gametic interpretation if the failure to show linkages were due to structural differences in the chromosomes of the parents. Alternatively, *FA* might have been considered in terms of a nonspecific mutagen with independent action on different factors. Further experiments have disqualified both of these views beyond reasonable doubt.

LT-7 served as an efficient donor and receptor of *FA* and was chosen for the study of the intrastrain transfers and to test these considerations (see table 1 for its markers). To be certain of the source of the *FA* employed, it was prepared (as described previously) without external bacterial or viral influences. *FA* was prepared from SW-184 (prolineless), SW-188 (methionineless), and SW-191 (leucineless). Each preparation was assayed for transduction from auxotrophy to

TABLE 3

The effect of FA from LT-7 and its derivatives upon LT-7 derivatives

CELLS/*FA*	LT-7	SW-184	SW-188	SW-191	BOILED *FA*
SW-184	203	26*	247	253	31*
SW-188	62	76	0	68	0
SW-191	198	210	236	18*	10*
LA-22 (control)	230	242	202	275	0

* Presumably spontaneous reverse mutations.

Figures are transductions from auxotrophy to prototrophy per plate.

prototrophy of each of the three LT-7 auxotrophs and LA-22 (control for the presence of any activity). The preparations had fairly uniform activity on LA-22. However, *FA* from each of the three LT-7 auxotrophs could transduce the other two but not its source culture (table 3). *FA* thus conforms to the genotype of the cells from which it comes. Several galactose-negative mutants were obtained in each of the three auxotrophs. None of several thousand transduced prototrophs was galactose positive. *FA*, from SW-184 (prolineless), when plated with SW-188 (methionineless) on minimal agar supplemented with proline, resulted only in proline independent colonies (prototrophs). Comparable results have been obtained with each of the three auxotrophs in similar experiments. In the course of transduction, there was no linked segregation or association of these three nutritional markers with each other or with fermentative markers. Streptomycin resistance provided still another marker that remained unaltered in cells transduced for other characters.

Several galactose-negative mutants were transducible to galactose-positive by *FA* from their parental wild type. *FA* from these mutants gave diverse results. The mutants were never transduced by their own *FA*, but they could be trans-

duced by *FA* from some of the other mutants. These interactions provided a basis for grouping the mutants with respect to allelism or genic identity (table 4).

All of the transductions discussed thus far have been in the direction of mutant to wild type. It is difficult, as a rule, to screen for changes in the other direction owing to the lack of adequate selective methods. This can be done with streptomycin resistance since the wild type condition is sensitive (S^s) and the mustant is resistant (S^r). Freshly harvested cells were exposed to *FA* from streptomycin resistant and sensitive "parents" and then plated upon EMB galactose. After two hours of incubation (to allow for phenomic lag, Davis 1950*a*) the plates were sprayed with a concentrated solution of streptomycin (0.1 g per ml). Table 5 shows that the transduction did occur but only when a

TABLE 4

The effect of FA from several galactose-negative mutants upon these same mutants

CELLS/*FA*	LT-7	GAL-1	GAL-2	GAL-3	GAL-4
Gal-1	+*	−†	−	+	+
Gal-2	+	−	−	+	+
Gal-3	+	+	+	−	−
Gal-4	+	+	+	−	−

* Galactose positive papillae produced.

† No more papillae than on control.

TABLE 5

Comparison of the effect of FA from streptomycin resistant and sensitive cells on sensitive cells

CELLS/*FA*	LT-7 (Gal +, S^s)	SW-514 (Gal +, S^r)	SW-191 (Gal −, S^s)	SW-515 (Gal −, S^r)
LT-7 (Gal+, S^s)	0	203 Gal+	0	174 Gal+
SW-191 (Gal−, S^s)	0	228 Gal−	0	158 Gal−

Figures are the number of streptomycin-resistant colonies per plate.

streptomycin resistant mutant was the source of the *FA* employed. No associated changes were found. The stability of the transduced cells was verified by tests of many daughter colonies by replica plating (Lederberg and Lederberg, 1952) to normal and streptomycin containing media.

It is now evident that the particular *FA* for which an assay has been defined is just one of several coexisting functions of a given filtrate. We are entitled to refer to *FA* for any of the genetic factors so far studied, and the range of action of a given filtrate can be designated in the same way as the genotype of the culture from which it is obtained: e.g., Prot, Gal+, Xyl+, S^r for SW-514 (figure 5), as well as for the *FA* derived from it. Unless otherwise qualified, however, *FA* will continue to refer to the transduction assayed on LA-22.

Adsorption of FA. The first step in transduction must be the adsorption of *FA*

on competent cells. LA-22 was harvested from nutrient agar plates. Aliquots were suspended in one ml of an active filtrate for various intervals. The cells were sedimented and plated on minimal agar to determine the number of exchanges. After a heat shock at 56 C to destroy any unsedimented cells, the supernatants were assayed with LA-22 for unadsorbed *FA*. Moderate amounts of *FA* were completely adsorbed within the time necessary for centrifugation (15 minutes) and were recovered quantitatively in the precipitated cells.

All tested smooth strains of *S. typhimurium* adsorbed *FA*. Cells of the donor strain adsorbed as efficiently as the others, consistently with the success of intrastrain transfers. Disinfection by boiling or ultraviolet irradiation (to leave an extremely small viable fraction) did not affect adsorption. Rough cultures, selected by aging in broth (Page *et al.*, 1951) did not adsorb. These results indicated that the site of adsorption is heat stable, is not affected by the death of the cell, and may be related to the somatic antigen.

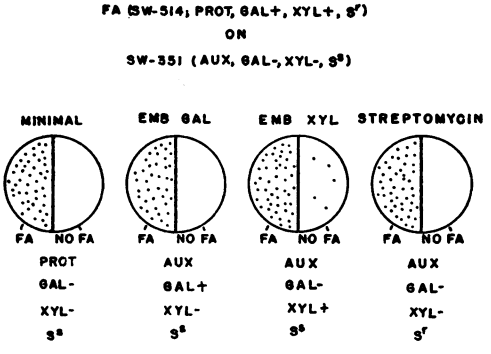


Figure 5. Multiple potentialities of an active filtrate

With the amounts previously used, *FA* assays were directly proportional to *FA* concentration. Cells of LA-22 were harvested from nutrient agar. Aliquots containing 10^{10} cells were sedimented in each of ten centrifuge tubes and the supernates discarded. Multiple aliquots of *FA* (one to ten ml) were added and 15 minutes at 37 C allowed for adsorption. Supernates and cells were collected and assayed on EML galactose. No concordant changes (i.e., galactose positive) were observed among the prototrophs. Figure 6 shows that a maximum number of transductions occurred with about eight ml of *FA*. The saturated sediments adsorb no more *FA* from larger aliquots. Except for a small systematic loss, probably mechanical, all units of *FA* are accounted for either in the supernatant or the sediment.

The interference in adsorption implied by saturation was demonstrated more explicitly in a blocking experiment. SW-188 (methionineless, M−) was exposed to an excess of *FA* from M− cells for fifteen minutes. *FA* from LT-7 (M+) was then added to the sedimented cells for an additional fifteen minutes before the cells were again sedimented. The M+ *FA* was not bound, nor was the SW-188 transduced. This verifies the blocking concept and indicates that the adsorption is irreversible after the fifteen minutes allowed for saturation.

Since adsorption of *FA* is so rapid it appears safe to assume that the large proportion of the individual bacteria are capable of adsorbing it. We can make an approximate minimum estimate of the number of adsorbable particles per ml of this filtrate by dividing the total number of bacteria by the number of ml required to saturate them (one particle per bacterium); $10^{10}/8$ or 1.3×10^9. A maximum number of particles per ml is set by the fact that the active filtrate showed no turbidity as might be expected with more than 10^{11} particles per ml.

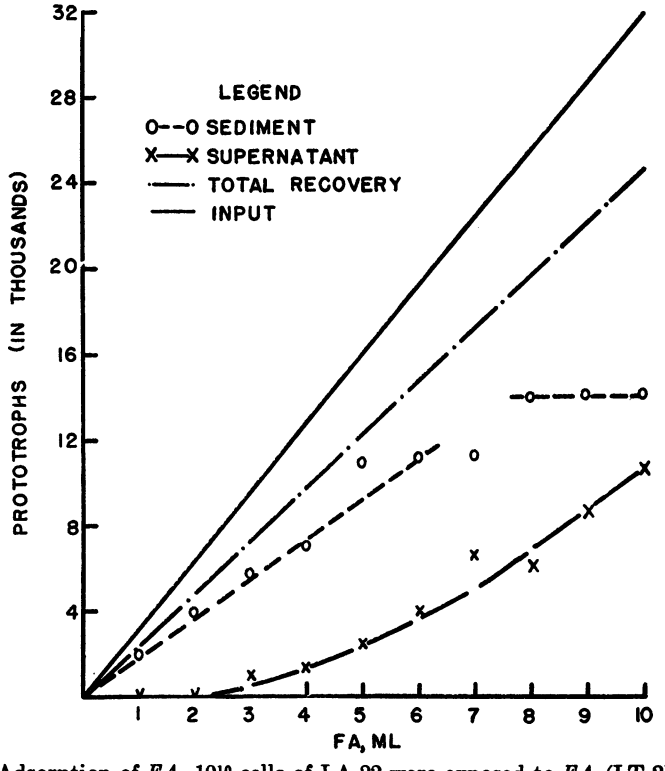


Figure 6. Adsorption of *FA*. 10^{10} cells of LA-22 were exposed to *FA* (LT-2) for 15 to 30 minutes. Supernatants and sediments were collected after centrifugation and assayed, respectively, for residual *FA* and for transductions already initiated.

So many unverified postulates are required that a detailed discussion of possible models for the kinetics of adsorption would be unprofitable here. It may be pointed out, however, that the low or zero frequency of double exchanges does not imply that one species of *FA* particle excludes another. If most of the bacteria are competent to be transduced, the frequency of a particular transduction will be the probability that any of the particles adsorbed will have a particular effect. Double transductions will occur in the same ratio to single exchanges as the absolute frequency of the latter, and this is too low (ca 10^{-5}) for double exchanges to be detected in our experiments. However, if transduction is limited to a small proportion of competent cells, dual transductions would

not have independent probabilities, and further assumptions such as mutual exclusion would be required to account for the low frequency of observed dual events.

The following picture appears to be most consistent with the observations to date. An active filtrate contains a population of numerous species of granules, each corresponding to a genetic effect although some may be intrinsically inert. Each bacterium may absorb a limited number of particles, in the possible range from one to perhaps one hundred. Each adsorbed particle has a fixed, independent probability of exerting its particular transductive effect. The low frequency of single, and particularly of double transductions, is limited by the total number of particles that may be adsorbed as well, perhaps, as by the low probability that an adsorbed particle will complete its effect.

Serial transduction. Dual transduction has never been observed in a single experiment. That this is due to the considerations described previously rather than some intrinsic limitation is shown by serial transfers. Once a cell has been transduced it can be grown out, reexposed to *FA*, and selected for other changes. SW-351 (Aux, Gal−, Xyl−) has been serially transduced from auxotrophy to prototrophy, from galactose negative to positive, and from xylose negative to positive. The order in which these transfers were accomplished made no difference. There was no loss of efficiency with the iterated transductions as compared to the single transduction of SW-351 for any of the characters.

Specificity of adsorption of FA. The adsorption experiments had indicated a correlation of adsorptive ability and immunological specificity. Preliminary experiments with some dozen *Salmonella* serotypes confirmed and narrowed this correlation to the presence of somatic antigen XII. Broth cultures of the serotypes to be tested were sedimented and one ml of *FA* was added. Adsorption proceeded for fifteen minutes, and then the reaction tubes were heat shocked at 56 C for one hour to sterilize the cells. Preliminary experiments with known adsorbing cells had shown that *FA* once adsorbed was not eluted by this procedure. The mixtures were assayed on LA-22 for free *FA*. Some fifty different serotypes have been tested in this manner. Although some types with XII are inert, none of the types without XII adsorbed. This correlation is maintained with the "*Salmonella coli*" types. The XII carrying strains that adsorbed were: *S. paratyphi* B, *S. typhi-murium* (25 strains), *S. stanley*, *S. heidelberg*, *S. chester*, *S. san-diego*, *S. abortus-ovis*, *S. typhi* W, *S. typhi* V, *S. enteritidis*, *S. moscow*, *S. blegdam*, *S. eastbourne*, *S. sendai*, *S. abony*, *E. coli* 3, *E. coli* 4, *S. kaapstad*, *S. salinatis*, *S. pullorum*, and *S. gallinarum*. The following XII types did not adsorb: *S. paratyphi* A and *S. abortus-bovis*, presumably owing to the absence of the XII_2 component. The nonadsorbing, non-XII types tested were: *S. typhi-murium* (rough variant), *S. cholerae-suis*, *S. newport*, *S. london*, *S. senftenberg*, *S. aberdeen*, *S. poona*, *S. worthington*, *S. hvittingfoss*, *S. kentucky*, *S. wichita*, *S. urbana*, *S. habana*, *S. altendorf*, *S. vejle*, *S. montevideo*, *E. coli* 1, *E. coli* 2, *E. coli* 5, *E. coli* K-12, *S. bonariensis*, *S. florida*, and *S. madelia*.

Inter-type transductions. It is not known whether the adsorption of *FA* is sufficient to indicate susceptibility to genetic transfer, but preliminary data

identify a possible receptor group, among which inter-type transductions may be possible.

S. typhi and *S. typhimurium* differ in a number of cultural and serological characters. The latter ferments both arabinose and rhamnose while the former does not ferment and is inhibited by either of these sugars. *S. typhi* Watson V was exposed to *FA* from *S. typhimurium* and inoculated into Durham fermentation tubes containing one per cent of either sugar in nutrient broth. After 24 hours a more luxuriant growth appeared in the *FA* treated cultures, and acid was produced by 48 hours. From these tubes cultures were isolated that differ from *S. typhi* only in their ability to ferment these sugars. The control cultures, without *FA*, show little evidence of growth and no evidence of fermentation. Although *S. typhimurium* produces gas from rhamnose and arabinose, these new forms remain typically anaerogenic. The experiment has also been conducted on agar. Treated cells were plated on EMB arabinose and EMB rhamnose. *S. typhi* occasionally mutates to a noninhibited form (Kristensen, 1948) which was represented by white papillae which were observed on both the experimental and control plates. However, the purple (fermenting) papillae were observed only on the experimental plates. Culturally they resembled the fermenting strains isolated after transduction in broth. These results have been repeated with two other strains of *S. typhi*. Using a streptomycin resistant mutant of *S. typhimurium* as the source of *FA*, it has been possible to transfer this character to *S. typhi*. Attempts to produce aerogenic fermentation of glucose by *S. typhi* by treatment with *FA* have all met with failure, possibly owing to insufficiently selective conditions to detect cells transduced for this character.

S. typhi is antigenically characterized IX, XII: *d*, —— (monophasic) while *S. typhimurium* is I, IV, V, XII: *i* — 1, 2, 3. *S. typhi* was exposed to *FA* from *S. typhimurium*, and transduction of the flagellar antigen was selected for. A tube based upon the mycological growth tube (Ryan *et al.*, 1943) was half filled with soft agar containing diluted anti-*d* serum (1/200 of serum titrating to 1/5,000). The cells were heavily inoculated at one side of the tube and watched for migration. In one experiment, two out of four experimental tubes showed migration while the three control tubes showed complete fixation of the inoculum. There was a sharp delineation between the migrating cells and the fixed inoculum. The former were fished from the uninoculated end of the tube and tested culturally and serologically. Both of the isolates culturally resembled *S. typhi*. One of them reacted with anti-*i* serum while the other did not react with either *S. typhi* or *S. typhimurium* flagellar antiserum and was diagnosed as a *j* phase (Kauffmann, 1936). The analysis of these two strains was confirmed by Dr. P. R. Edwards. Transduction of the *i* antigen was obtained from twelve of thirty-one tested inocula of 10^8 *FA* saturated *S. typhi* cells. "*j*" phases have appeared occasionally in both experimental and control tubes. No *i* phases were detected in 50 control tests without *FA*. The complete antigenic analysis of the "hybrids" is IX, XII: *i*, ——. Unlike *S. typhimurium*, from which the *i* flagellar phase was derived, phasic variation has not been found in these "hybrids". Experiments are now in progress seeking transduction of other flagellar and somatic antigens.

The transduced cell. Prototrophs produced by transduction [*FA* (LA-2) on LA-22] have been tested for their stability both in vegetative reproduction and further transduction. After isolation from the experimental plate they were purified by streaking. Five single colonies were grown in complete broth and plated. Two hundred colonies from each were picked and retested on minimal agar: all were prototrophic. The transduced culture was reexposed to *FA*, and another change was selected (galactose negative to positive). Of some 1,500 colonies tested by replica plating, all retained the initial transduction to prototrophy.

The transduced culture does not release *FA* during its growth nor is *FA* obtainable from it by any other means than those employed for the parent culture. Some difficulty has been encountered in this respect with the products of intrastrain transduction. They were all resistant carriers of the phage associated with active filtrates and some new phage was needed to evoke *FA* from them. Phage resistance also reduces the efficiency of iterated transduction, presumably because of impaired adsorption of *FA*.

Spontaneous reverse-mutations regain the ability to transduce their mutant parents as do transduced reversions. That is, when a cell goes from A− to A+ by either means, it can again produce A+ agent. Mutation in free *FA* has not yet been studied.

The relationship between bacteriophage and FA. Several recent convergent lines of evidence point to the identity of *FA* particles and bacteriophage. *FA* and phage have a common filtration end point; ninety-nine per cent of both are retained by a membrane of A.P.D. 120 mμ. They have a common specificity of adsorption on *Salmonella* serotypes, correlated with somatic antigen XII. In adsorption on *S. typhimurium* both reach saturation at the same point, and the phage to *FA* ratio remains constant. During the course of purification, *FA* and phage remain together. In short term experiments, *FA* and phage are released simultaneously from phage infected bacteria. Electron micrographs show a morphological similarity of particles of proper size.

That the phage particle can be only a passive carrier of the transductive genetic material is shown by the following experiments. From single phage particles grown on bacterial cells there are obtained high titered phage and a population of *FA* encompassing the entire genotype of the parental cells but capable of only one transduction per bacterial cell. Single phage particles, from this filtrate, can be grown on bacterial cells from the same original parent but of different genotype. The *FA* produced is comparable to the genotype of the secondary donor.

In the section on the evocation of *FA*, mention was made of the apparent regeneration of *FA* by transfer. This was explained as being due to the association of *FA* with phage which served to continuously stimulate its production. To test this, A−, B+, C+ cells were treated with penicillin. The filtrate was transferred with the same cells to yield *FA* (A−, B+, C+) and a phage which could be assayed on these same cells. When added to A+, B−, C+ cells (from the same original parent), the *FA* obtained was A+, B−, C+. All of the B+ agent was adsorbed and lost, and agents paralleling the genotype of the B−

cells obtained. *FA* had not propagated as such but rather was associated with the necessary stimulus for further production, the phage.

DISCUSSION

Genetic exchange in *S. typhimurium* is mediated by a bacterial product which we have called *FA* (filtrable agent). An individual active filtrate can transfer (transduce) many hereditary traits from one strain to another. Although the total activity of this filtrate encompasses the genotype of its parental culture, each transduction transmits only a single trait per bacterium. This contrasts with genetic exchange in *E. coli*, strain K-12, where there is unrestricted recombination of the several markers that differentiate two parental lines.

FA may be considered as genetic material which enters the fixed heredity of the transduced cell. We may ask whether this transfer is a simple super-addition or a substitutive exchange and replacement of the resident genetic factors. If streptomycin resistance is a recessive mutation, as inferred from studies of heterozygous diploids in *E. coli* (Lederberg, 1951*b*), the transduction of resistance disqualifies the simple addition mechanism.

Two aspects of *FA* must be carefully distinguished: the biological nature of the particles themselves and their genetic function. There is good reason to identify the particle with bacteriophage. Nevertheless, the phage particle would function as a passive carrier of the genetic material transduced from one bacterium to another. This material corresponds only to a fragment of the bacterial genotype. For example, when *FA* from a marked prototroph is plated with an auxotroph on minimal agar, the genotype of the presumed "donor nucleus" is not observed among the transduced prototrophs. The hypothesis of *FA* as a genetic complex rather than a unit might be maintained if the singular effects produced depended on a small chance of release of any particular activity from a complex particle or on some localized nonheritable happenstance in the cell that ordinarily left only one function sensitive to transduction. Still the originally singly transduced cell develops as an isolated clone. Since the clone is composed of some 10^7 bacteria, one might expect that a complex residuum of an *FA* particle, if viable, would transduce some one of the daughter cells for another character during the growth of the clone. However, each *FA* particle produces only a single transduced clone. This speaks for the simplicity of its constitution as well as of its genetic effect.

When LA-22 is transduced from auxotrophy (phenylalanineless and tyrosineless; tryptophanless) to prototrophy, we have an apparent dual change. If this mutant is plated on minimal agar supplemented with phenylalanine and tyrosine, it occasionally reverts to the first step auxotrophic condition. However, when LA-22 is transduced on this medium, no more first step auxotrophs are found than can be explained by spontaneous reversion. The majority of the selected colonies are prototrophs. We have not been able to affect more than one trait in any other inter- or intrastrain transductions. It seems likely that the nutrition of LA-22 was determined by two successive mutations at the same genetic site. Davis' (1951) scheme for aromatic biosynthesis corroborates this notion. Al-

though the mutant LA-22 can revert spontaneously to an intermediate allele, transduction brings about a substitution of the wild type gene for full synthesis.

The most plausible hypothesis for the *FA* granules is that they are a heterogeneous population of species each with its own competence—in other words, each carries a "single gene" or small chromosome fragment.

Regardless of the nature of the *FA* particles, some mechanism must be postulated for the introduction of the transduced genetic material to the fixed heredity of the recipient cell. Muller's (1947) analysis of type transformation in the pneumococcus is apropos here: "... there were, in effect, still viable bacterial chromosomes, or parts of chromosomes, floating free in the medium used. These might, in my opinion, have penetrated the capsuleless bacteria and in part taken root there, perhaps, after having undergone a kind of crossing-over with the chromosomes of the host."

In a preliminary report on the *Salmonella* recombination system (Lederberg *et al.*, 1951) it was suggested that *FA* might be related to bacterial L-forms (Klieneberger-Nobel, 1951). The occurrence of swollen "snakes", filtrable granules, and large bodies in response to certain agents is characteristic both of *FA* and L-forms. Except for the suggestion of viable filter passing granules we have not repeated the reported cycles. The visible agglutinable granules and the antiserum-induced swollen form are not necessary for *FA* activity. However, this failure to fit all of the elements to a simple scheme may be due to a system more complex than we are now aware.

The bacteriological literature has numerous reports of results which might be interpreted as transduction (see reviews by Luria, 1947, and Lederberg, 1948). These experiments have been criticized or neglected because of difficulties in their reproduction and quantitization but might now be reinvestigated in light of the findings presented. A citation of some of the more pertinent ones should suffice at this time. Wollman and Wollman (1925) reported the acquisition of *Salmonella* immunological specificity by *E. coli* via filter passing material. Similar material (which can be obtained by phage lysis) has been implicated in the change of penicillin resistant staphylococci and streptococci to relative penicillin sensitivity (Voureka, 1948; George and Pandalai, 1949). *Shigella paradysenteriae* (Weil and Binder, 1947) acquired new immunological specificity when treated with extracts of heterologous types. Boivin (1947) reported a similar change in *E. coli*. Unfortunately his strains have been lost and confirmation is impossible. Bruner and Edwards (1948) in a report of variation of somatic antigens of *Salmonella* grown in the presence of specific serum commented on the possibility that bacterial products dissolved in the serum were responsible for the changes.

These systems, provocative as they are, are insufficiently documented for detailed comparison with *Salmonella* transduction. The transformations in the pneumococcus (Avery *et al.*, 1944; McCarty, 1946) and *Hemophilus influenzae* (Alexander and Leidy, 1951) have been studied more completely.

The genetic "transformation" of the capsular character of the pneumococcus depends on a specific bacterial product (pneumococcus transforming principle,

PTP). Originally interpreted as a directed mutation, it is now regarded as a variety of genetic exchange (Ephrussi-Taylor, 1950). Thus far transformations have been achieved for the full capsular character (Griffith, 1928), a series of intermediate capsular characters (Ephrussi-Taylor, 1951), M protein character (Austrian and MacLeod, 1949), and penicillin resistance (Hotchkiss, 1951). As in *Salmonella* each character is transformed independently. However, there are several differences between the two systems. *FA* must be evoked while the *PTP* is extractible from healthy cells. The resistance of *FA* to various chemical treatments has given only negative evidence of its chemical nature. The role of desoxyribonucleic acid in the *PTP* was verified by its inactivation by desoxyribonucleic acidase. Retention of activity by gradocol membranes has given comparable estimates for the size (about 0.1 μ) of the *FA* particles affecting two different characters. On the other hand, while the particle size of the *PTP* has been variously estimated from an average centrifugal mass of 500,000 (Avery *et al.*, 1944) to an ionizing irradiation sensitive volume equivalent to a molecular weight of 18,000,000 with high asymmetry (Fluke *et al.*, 1951), it is considerably smaller than the *FA* particle. Pneumococci must be sensitized by a complex serum system for adsorption of *PTP*. The low but poorly determined frequency of transformations has been thought to be due to the low competence of the bacteria. In the absence of adsorption experiments a system similar to *Salmonella* has not been ruled out. Important information is still lacking in both systems and time may resolve these apparent differences.

The relationship of transduction in *Salmonella* to sexual recombination in *E. coli* is obscure. Transduction has not been found in crossable *E. coli* nor sexual recombination in *Salmonella*. These genera are extremely closely related taxonomically but seem to have entirely different modes of genetic exchange.

Sexual recombination was first demonstrated in *E. coli*, strain K-12. With the development of an efficient screening procedure, two to three per cent of *E. coli* isolates were proved to cross with strain K-12 (Lederberg, 1951*a*). The agent of recombination in *E. coli* is almost certainly the bacterial cell. The cells apparently mate, forming zygotes from which parental and recombinant cells may emerge following meiosis, in which linkage is a prominent feature (Lederberg, 1947). The combination of genomes within a single cell has been confirmed by the exceptional occurrence of nondisjunctions which continue to segregate both haploid and diploid complements (Zelle and Lederberg, 1951). Although lysogenicity plays a critical role in transduction in *Salmonella*, all combinations of lysogenic and nonlysogenic cultures of *E. coli* cross with equal facility (Lederberg, E. M., 1951).

Owing to the lack of recombination of unselected markers, transduction is a less useful tool than sexual recombination for certain types of genetic analysis. However, as *FA* may correspond to extracellular genetic material, such problems as gene reproduction, metabolism, and mutation may be more accessible to attack. Sexual systems usually provide for the reassortment of genetic material and given an important source of variation for the operation of natural selection in organic evolution. Both sexual recombination and transduction, because of

their low frequency, allow only limited gene interchange in bacteria. Transductive exchange is limited both in frequency and extent.

It is too early to assess the role that transduction may have played in the development of the immunologically complex *Salmonella* species. White (1926) speculated that the many serotypes evolved by loss variation from a single strain possessing all of the many possible antigens. Bruner and Edwards (1948) obtained specific examples of loss variation with contemporary species. Transduction provides a mechanism for transfer of some of the variation developed spontaneously and independently between the "descending" lines. The genus *Salmonella* includes a group of serotypes which share a receptor for *S. typhimurium FA*. Other receptor groups have yet to be sought. Within such groups it should be possible to evolve in the laboratory other new serotypes comparable to the antigenic hybrid of *S. typhi* and *S. typhimurium*.

Several different bacterial genera have been intensively studied with regard to modes of genetic exchange. Each of the several known systems differs in details that enlarge our notions of bacterial reproduction and heredity.

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SUMMARY

When *Salmonella typhimurium* is grown in the presence of a variety of mildly deleterious agents, especially weakly lytic phages, it produces a filtrable agent (*FA*) capable of transferring hereditary traits from one strain to another.

Individual filtrates may transduce many different traits, but no more than one in a single bacterium. The activities of a filtrate parallel the characteristics of the donor cells. Nutritional, fermentative, drug resistance, and antigenic characters have been transduced. The new characters are stable after many generations of subcultures.

FA is resistant to such bacterial disinfectants as chloroform, toluene, and alcohol and to such enzymes as pancreatin, trypsin, ribonuclease, and desoxyribonuclease. The size of the *FA* particle, as determined by filtration through gradocol membranes, is about 0.1 micron. Adsorption of *FA* is rapid and, among various serotypes tested, is correlated with the presence of somatic antigen XII.

The maximum frequency of transduction for any one character has been 2×10^{-6}, a limit set by saturation during adsorption. Some inter-type transfers have been observed. For example, the *i* flagellar antigen from *Salmonella typhimurium* has been transduced to *S. typhi* to give a new serotype: IX, XII; *i*, —. Genetic transduction in *Salmonella* is compared and contrasted with "type transformation" in *Hemophilus* and the pneumococcus and with sexual recombination in *Escherichia coli*.

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