[13] We wish to thank Dr. R. L. Sinsheimer of the California Institute of Technology for his interest and suggestions along this line.

[14] Ingram, V. M., *Nature*, **180**, 326 (1957); Hunt, J. A., and V. M. Ingram, *Nature*, **181**, 1062 (1958).

[15] It should be made clear that here "mutation" does not include gross or direct effects of mutagenic agents on DNA or chromosomes to produce deletions, inversions or other abnormalities which render a particular gene inactive or modify its phenotype effect. It seems reasonable for purposes of *study of the induction process* to divide "mutations" into at least two classes: (1) those mutations probably involving one DNA nucleotide pair and exerted through the process of gene replication and (2) those mutations involving one or *more* nucleotide pairs exerted finally through some physical or chemical action on DNA (or RNA) and *not* through gene replication. In the case of UV-induced mutation it appears that the former by far exceed in number the latter (if they occur at all). Further this is not to suggest that one nucleotide pair may somehow "code" a particular amino acid but rather that a change in the nucleotide composition of the nucleotide pair unit in a particular *sequence* may lead to a change of one amino acid originally specified by the particular sequence involved to another amino acid.

*TRANSDUCTION BY STAPHYLOCOCCAL BACTERIOPHAGE**

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Communicated by Joshua Lederberg, March 27, 1959

Transduction of genetic material has been reported previously for *Salmonella* and *Escherichia coli* bacteriophages.[1] It is the purpose of this communication to call attention to gene transfers accomplished by staphylococcal bacteriophage.

Materials and Methods.—Preliminary studies suggested that among the staphylococcal typing phages examined,[2] NCTC 8406 (phage 53) and its propagating bacterial strain NCTC 8511 would be suitable for study of gene transfer between staphylococcal cells. Phage 8406 forms plaques well on 8511 and an appreciable number of the 8406–8511 complexes result in lysogeny of the 8511 cells. Such lysogenic cells formed are subsequently induced to produce phage by ultraviolet radiation.[3] Because of these advantageous features a variety of bacterial mutants was obtained in the 8511 line, and selected for further study.

Cultures were grown in 10 ml volumes of Difco nutrient broth on a rotating (28 rpm) circular test tube rack at 37C. Cell clumping which can be a problem with staphylococci, was controlled by allowing the cultures to grow in a stationary position for 10–12 hours, followed by 4–5 hours of growth in the rotating rack. This procedure usually produces cultures with about 2×10^9 cells per ml in which 50 per cent of the cells are single, 46 per cent are either in pairs or are single cells about to divide, and the remaining 4 per cent occur chiefly as clamps of 3–4 cells. Ultraviolet inactivation of these cultures is not appreciably different from a one-hit process to a 1 per cent survival level.

Phage lysates were prepared by UV induction of cultures lysogenic for 8406 phage. Cultures were removed from the rotator and suspended in saline or phosphate buffer solutions at a density of about 5×10^8 cells per ml and irradiated with ultraviolet to give 1.0–0.1 per cent survival. Concentrated nutrient broth (10×) was

added (1 volume/10 volumes) to the irradiated suspension and the mixture rotated for 4–6 hours at 37C, after which it was left on the bench at about 20 C for 16 hours. During this latter period the cultures usually cleared. Cleared cultures had from 2×10^9 to 10^{10} plaque particles per ml on 8511. The lysates were sterilized by filtration through Mandler filters.

Transduction experiments were performed by mixing phage with phage-sensitive cells at multiplicities of one phage or less per cell and allowing the mixture to incubate at 37C for 5–25 minutes, during which time 95 per cent of the added phage adsorbed to the cells. Samples of 0.1–0.8 ml were added to 2 ml volumes of melted 0.6 per cent agar (50C) and poured on either nutrient agar or on agar indicating acid production from carbohydrates.[4] Selection was made for transduction of genes for drug resistance characters,[6] and to accomplish this it was necessary to overcome 4–6 hours of phenotypic delay.[5] The agar pour platings were overlayed routinely with melted 0.6 per cent agar containing drug within one hour of plating to serve as a control, and after four hours, to enumerate transductions.

Results.—Transduction of the genes for resistance to streptomycin and novobiocin was observed. The data given in Table 1 show that streptomycin-resistant clones

TABLE 1

TRANSDUCTION OF THE GENES FOR RESISTANCE TO STREPTOMYCIN AND NOVOBIOCIN[6]

Expt.	Phage Source	Amount of Phage $\times 10^9$	Drug-Resistant Clones Produced S^r	N^r
50	N^s	7.7	...	0
	N^r	8.8	...	680
51	N^sS^s	1.9	0	20
	N^rS^r	4.0	1,210	1,100
78	S^s	3.9	0	
	S^r (1)	4.6	290	
	S^r (2)	3.1	330	
TG-61	N^s	2.6	...	10
	N^r	8.5	...	300

are formed only when streptomycin-resistant cultures (S^r) are the source of phage. Larger numbers of novobiocin-resistant clones (N^r) were found on plates with phage from N^r sources than from novobiocin sensitive (N^s) sources. Since platings containing no phage have small numbers of N^r, we believe the source of the novobiocin-resistant clones on the platings from N^s sources to be spontaneous reversions.

The number of drug-resistant clones produced by transduction is proportional to the amount of phage employed (Fig. 1).

The phenotypic expression of the tranduced S^r genes is accomplished in 4–6 hours, while approximately 50 per cent of the transduced N^r genes appear to achieve phenotypic expression immediately upon entering the cell (Fig. 2). The immediate expression of N^r would suggest that N^r is dominant to N^s, however the delayed N^r require some other explanation, such as delay in penetration of the cell. It is doubtful that this delay would be of 4–5 hours' length. Alternately, the behavior of the transduced N^r genes may indicate that novobiocin is a slowly acting drug, requiring 3 hours for its killing effect. During such a time the phenotypic expression of transduced resistance genes might be achieved.

The data indicate that approximately one phage particle in 10^7 to 10^8 carries a gene for streptomycin or novobiocin resistance.

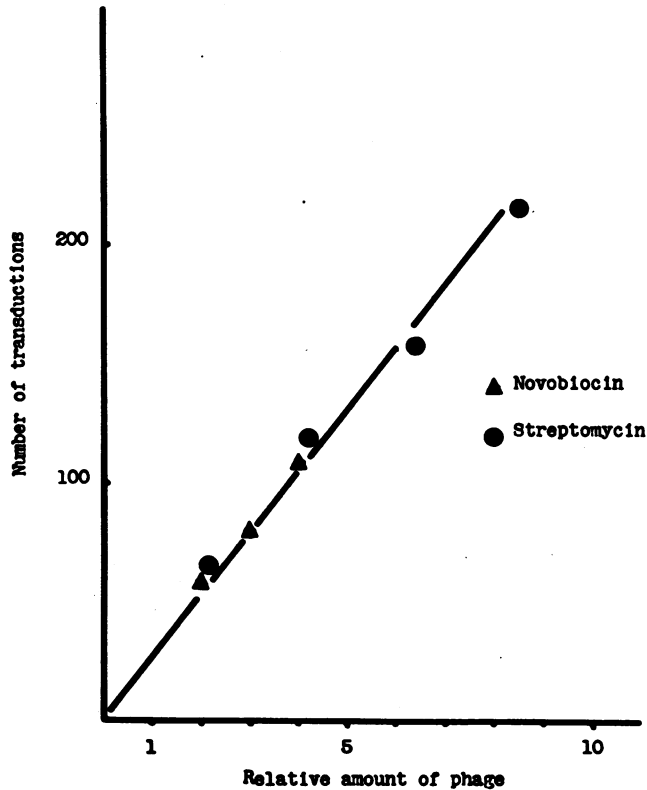

FIG. 1.—Proportionality between number of transductions and amount of phage. Different lysates: relative amount 1 for streptomycin equals 4.5 × 10^8 phage particles; relative amount 1 for novobiocin equals 1.7 × 10^9 phage particles. The frequencies of transduction are: S^r, one per 2 × 10^7; N^r, one per 6.8 × 10^7.

The data given also indicate that about one cell in 10^7–10^8 exposed to a phage preparation yields a cell of changed genotype. Such a low frequency virtually eliminates the detection of coincidental transductions and any clones altered for more than a single genetic character can be considered to indicate the transduction of closely linked genes. Genetic linkage has been looked for by using multiply marked stocks, for example: $S^rN^rMan^-Lac^+$ (donor phage source) $-X$ (applied to:) $S^sN^sMan^+Lac^-$ (phage-sensitive recipient cells).[6] The S^r or N^r produced by transduction were examined for changes of the other distinguishing genetic markers. No evidence for linkage has been found between any of the following markers: *S*, *N*, *E*, *Lac*, *Man*, with at least 300 transductions per test.

Of 462 transductions, 461 were found lysogenic with respect to the parent NCTC 8511 strain, evidence that phage is the vector or co-vector of genetic transfer. The remaining culture does not produce a plaque-forming phage and is non-sensitive to exogenous phage and may therefore carry a defective prophage. The complexity of the situation with regard to phage deserves additional comment. The host culture NCTC 8511 and its derivatives are lysogenic for a phage which

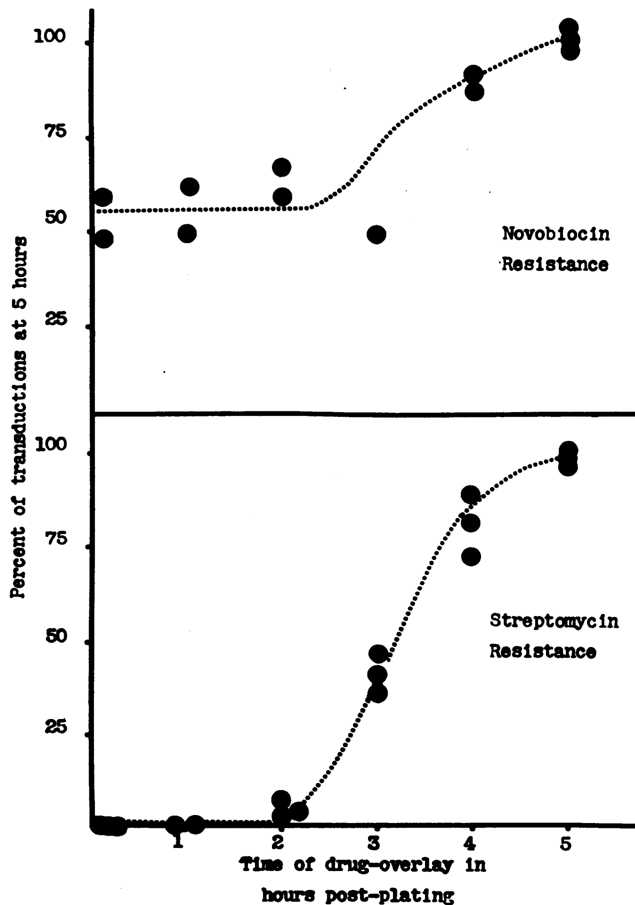

FIG. 2.—Phenotypic expression of transduced genes. The maximum overlay time was 5 hours post plating. At this time approximately 90 per cent of transductions have been phenotypically expressed.

attacks NCTC 8325, the propagating strain of typing phage 47.[7] There is good evidence also for a third phage in our lysates.[8] These phages have been found at low titers in our transducing lysates (10^{-4}–10^{-6} of the phage-forming plaques on 8511). Direct test of the phage attacking NCTC 8325 failed to show transduction of streptomycin resistance between cells of the 8511 line by this phage. Because of the low incidence of these phages in our lysates, and the negative results with one of them in transduction experiments, we ascribe our transduction to the major phage constituent of our lysates, phage 8406 (53).

In a transduction system in *E. coli* the formation of partially diploid clones (heterogenotes), as a result of transduction, was noted.[9,10] UV-induction of these heterogenotic cultures yielded lysates giving high frequency of transduction, because most phage particles carried bacterial genetic material. Examination of the transductional clones formed by staphylococcal bacteriophage failed to show a heterogenotic condition for either Man or Lac. Lysates of 4 N^r clones and 4 S^r

clones produced by transduction gave a low frequency of transduction of N^r and S^r (one transduction per 10^7–10^8 phage). Staphylococcal bacteriophage transductions therefore appear to resemble those found in Salmonella by Zinder and Lederberg.

It is possible with the genetically labeled strains used for transduction studies to test for sexual recombination between cells of the NCTC 8511 strain. This can be done by selection for double drug resistance, each of the parent cells contributing one resistance gene, and by the use of two or more unselected genetic markers:[11] $N^rS^sMan^+Lac^-$ crossed with $N^sS^rMan^-Lac^+$ yields $N^rS^rMan^-Lac^-$ and $N^rS^rMan^+ Lac^+$ combinants for double drug resistance (selected) in which recombination for the fermentation markers (unselected) has occurred. Using various combinations of *N*, *S*, *E*, *Lac*, *Man* and *Ly*, we have made limited tests for sexual recombination in our strains. Although strains doubly resistant to drugs were obtained, these were always non-recombinant for unselected markers (Table 2). The most plausible origin of these doubly resistant strains is spontaneous mutation. In support of this explanation was the association between the occurrence of doubly resistant strains and the mutabilities of the loci for drug resistance. Qualitatively the mutability order is $S < E < N$. Doubly drug resistant clones were obtained most frequently in combinations of the more mutable loci (*N*, *E*) and the clones most often resembled the more mutable "parent."

TABLE 2

DOUBLY DRUG-RESISTANT CLONES PRODUCED IN MIXED GROWTH OF SINGLY RESISTANT MUTANTS OF STAPHYLOCOCCI[6]

Expt.	Genotypes in Mixed Growth: Strain 1	Genotypes in Mixed Growth: Strain 2	Resistance Markers Selected	Non-selected Markers	Non-selected Markers Observed	Suspected Reversion of Strain*
1	$N^sS^rLac^-Ly^+$	$N^rS^sLac^+Ly^-$	N^rS^r	*Lac*, *Ly*	37 Lac^-Ly^+	1
2	$N^sS^rLac^-Man^+Ly^+$	$N^rS^sLac^+Man^-Ly^-$	N^rS^r	*Lac*, *Man*, *Ly*	58 $Lac^-Man^+Ly^+$	1†
3	$S^rE^sLac^-Man^+Ly^+$	$S^sE^rLac^+Man^-Ly^-$	S^rE^r	*Lac*, *Man*, *Ly*	4 $Lac^-Man^+Ly^+$	1
					6 $Lac^+Man^-Ly^-$	2
4	$S^rE^sLac^-Man^+Ly^+N^s$	$S^sE^rLac^+Man^-Ly^-N^r$	S^rE^r	*Lac*, *Man*, *Ly*, *N*	18 $Lac^-Man^+Ly^+N^s$	1
					2 $Lac^+Man^-Ly^-N^r$	2
5	$N^rE^sLac^+Man^-S^s$	$N^sE^rLac^-Man^+S^r$	E^rN^r	*Lac*, *Man*, *S*	35 $Lac^-Man^+S^r$	2
					4 $Lac^+Man^-S^s$	1

* The non-selected markers are derived from one or the other strain in the growth mixture and therefore suggest that spontaneous reversion was the mechanism by which the doubly drug-resistant strains were formed. For example, in experiment 1, $N^sS^rLac^-Ly^+$ yields $N^rS^rLac^-Ly^+$ by spontaneous reversion of N^s.
† Three experiments, in one of which inclusion of F^+ *E. coli* failed to influence the number or kind of doubly resistant staphylococci produced in the mixture.

Discussion.—The evidence that has been presented indicates that staphylococcal bacteriophage preparations (of typing phage 53) can transfer staphylococcal genetic material. The limitations on the number of genetic markers found transferred is owing to the lack of genetic factors for which selection can be exercised. Since the nutrition of our strains is nearly defined,[8] it is expected that requirements for amino acids and vitamins, and the utilization of carbohydrates will become available shortly for further study of this transduction system. It is hoped that the medically

important staphylococcal characteristics such as hemolysin and coagulase production will become available for analysis, either directly through selection or by means of linkage to other selectable markers. More details of the above and the results of further experiments will be reported elsewhere.

Summary.—Genetic transduction by staphylococcal bacteriophage has been demonstrated. About one phage particle in 10^7–10^8 transfers the genes for resistance to streptomycin and novobiocin.

* Department of Biophysics Paper No. 83. This study supported by grants from the National Institute for Allergy and Infectious Diseases, Public Health Service (E-1345C1) and the National Science Foundation (NSF-G5861).

[1] Zinder, N. D., *Cold Spring Harbor Symp. Quant. Biol.*, **18**, 261–269 (1953); Lederberg, J., *Am. Scientist*, **44**, 264–280 (1956); Hartman, P., *The Chemical Basis of Heredity* (Baltimore: Johns Hopkins Press, 1957, pp. 408–467.)

[2] National Collection of Type Cultures (NCTC), London, received in lyophilized form, of the following phages have been examined: 9300(42F); 8429(55); 8413(29); 9788(80); 8406(53). Excepting NCTC 8406, these phages were either difficult to grow, or non-lysogenizing (lytic) phages.

[3] Weigle, J. J., and M. Delbrück, *J. Bact.*, **62**, 301–318 (1951).

[4] Morse, M. L. and M. L. Alire, *J. Bact.*, **76**, 270–271 (1958).

[5] Davis, B. D., *Experientia*, **6**, 41–50 (1950).

[6] Symbols are *N*, Novobiocin; *S*, Streptomycin; *E*, erythromycin; *Lac*, lactose fermentation; *Man*, mannitol fermentation; *Ly*, lysogenicity. Superscripts: *r*, *s*, resistant and sensitive respectively; $^+$, $^-$, ability to ferment and inability to ferment carbohydrates, except in the case of *Ly*, where $^+$ signifies lysogenic, $^-$ signifies nonlysogenic and sensitive to exogenous phage. The parent staphylococcal strain is inhibited by 1 mcg/ml streptomycin and 0.05 mcg/ml novobiocin. The resistant strains are not inhibited by 25 mcg/ml and 2 mcg/ml, respectively. The concentration of the drugs in the overlay agar were: *S*, 100 mcg/ml; *N*, 20 mcg/ml.

[7] Unpublished observations and a personal communication from Dr. P. M. Rountree.

[8] Unpublished observations, John Mann, this laboratory.

[9] Morse, M. L., E. M. Lederberg, and J. Lederberg, *Genetics*, **41**, 142–156 (1956).

[10] Morse, M. L., E. M. Lederberg, and J. Lederberg, *Genetics*, **41**, 758–779 (1956).

[11] Lederberg, J., *J. Bact.*, **59**, 211–215 (1950).

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*ABERRANT TETRADS IN SORDARIA FIMICOLA**

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Communicated by B. O. Dodge, March 31, 1959

In an earlier paper[1] describing three ascospore color mutants in the homothallic pyrenomycete *Sordaria fimicola*, the rare occurrence of unusual tetrads with unexpected ratios of wild-type and mutant ascospores was noted for all three loci. In the course of further studies of two of these mutant loci, a number of asci showing aberrant tetrads were isolated and analyzed in an effort to obtain a clearer idea of the mechanism responsible for their occurrence. Such tetrads in yeast had previously been explained by Lindegren[2] as resulting from "gene conversion" in a heterozygote, usually involving a change of the dominant gene to its recessive allele. Later, Mitchell[3] reported similar abnormal segregations for the pyridoxine locus in *Neurospora*, which she explained as probably resulting from double replication.