Reversion of Transduced Antigenic Characters in *Salmonella typhimurium*

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SUMMARY: A strain of *Salmonella typhimurium*, in which the H antigens had been altered by transduction with a bacteriophage lysate of *S. abony* to the form $b \leftrightarrow ena$ has been found to revert in the first or second phases giving rise to organisms with the structure $i \leftrightarrow ena$ and $b \leftrightarrow 1.2$. No reversions in both phases have so far been detected. This phenomenon has been observed only in non-lysogenic strains; lysogenic varieties have remained stable.

Transduction by bacteriophage of antigenic characters in the salmonella group was first reported by Zinder & Lederberg (1952); a more detailed study of the subject was published by Lederberg & Edwards (1953). It was shown that flagella antigens could be freely transferred between members of groups A, B, and D of the Kauffmann-White scheme, Phase I antigens of the donor being transduced to the Phase I locus of the recipient, and Phase II antigens, independently, to the Phase II locus. Many salmonella types were 'synthesized' by transduction, including several not yet observed in nature. The synthetic types were carefully examined for any sign of the continued presence of the recipient's original H antigen, and none was found. The new types appeared as stable as naturally occurring salmonellae, and no reversions to the parent type were discovered.

Later Edwards, Davis & Cherry (1955) were able to extend these studies to other groups of the Kauffmann-White scheme; they likewise did not report any reversions in their transformed cultures. It has therefore been assumed that the genetic factor controlling the H antigen has been completely replaced by the transduced gene, and that the original fragment has been eliminated. It is the purpose of this note to give an account of observations on some salmonella H antigens which have been changed by transduction, and subsequently reverted to the parent form.

METHODS

The main salmonella strains used are listed in Table 1. All were obtained from Dr B. A. D. Stocker and Dr C. Quadling of the Lister Institute, London, S.W. 1, and those whose reference initials are SL had been prepared by them.

The phage used throughout for transduction was P 22, as described by Zinder & Lederberg (1952). None of the salmonella strains in Table 1 carried P 22, the SL series having been specially prepared to act as donors in experiments on transduction.

The rabbit sera used for identifying antigens were those prepared for general use by the Standards Laboratory, Colindale. In serum selection experiments

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using semi-solid agar (Edwards & Bruner 1942) the sera were used at a concentration about 200 times their agglutinating titre. Antigenic variants were isolated by inoculating a thick suspension of the culture on semi-solid agar containing antiserum to the H antigens other than those sought. When verifying the phases of an organism, serum selection was done in Craigie tubes

Table 1. *Origin and antigenic type of salmonella strains used*

Ref. No.	H antigens		O antigens (antigen I omitted)	Origin and remarks
	Ph. I	Ph. II		
LT2	<i>i</i>	1.2	4.5.12	A wild strain of <i>Salmonella typhimurium</i> (Zinder & Lederberg, 1952)
SL270	<i>i</i>	1.2	4.5.12	A double mutant of LT2, requiring methionine and tryptophan
SW803	<i>b</i>	<i>enx</i>	4.5.12	A strain of <i>S. abony</i> catalogued as no. 103 by Edwards & Bruner (1942)
SL113	<i>b</i>	1.2	4.5.12	LT2 altered by transduction with a P22 lysate of SW803. Prepared Nov. 1953
SL141	<i>b</i>	<i>enx</i>	4.5.12	SL113 altered by transduction by the same lysate of SW803. Prepared Jan. 1954
SL147	<i>b</i>	<i>enx</i>	4.5.12	A streptomycin resistant mutant of SL141, isolated by selection in Jan. 1954
SL150	<i>b</i>	<i>enx</i>	4.5.12	An azide resistant mutant of SL147, isolated by selection in Feb. 1954
SW715	<i>r</i>	1.2	4.5.12	A strain of <i>S. heidelberg</i> catalogued as No. 16 by Edwards & Bruner (1942)
SW541	(<i>i</i>)	(1.2)	4.12	A non-motile strain of <i>S. typhimurium</i> . (Stocker, Zinder & Lederberg 1953)
SW543	(<i>b</i>)	(—)	4.5.12	A non-motile monophasic strain of <i>S. paratyphi</i> B. (Stocker, <i>et al.</i> 1953)

(Mackie & McCartney, 1953). For transduction, 1 ml. of a thick suspension (*c.* 1×10^{10} organisms) of an overnight culture was mixed with 1 ml. of a high titre phage preparation, incubated at 37° for $\frac{1}{2}$ hr., centrifuged, and the deposit plated on semi-solid agar containing the appropriate serum.

Standard techniques as given by Adams (1950) were used in all work involving bacteriophage.

RESULTS

The main experimental findings are summarized in Table 3 which gives details of the most important strains tested for reversion of transduced antigenic characters to the parent type. The first observations were made in October 1956, when strain SL150 (see Table 1) was plated on nutrient agar and the resulting overnight growth was, as a control in another experiment,

inoculated heavily on to a semi-solid agar containing antiserum to factors *b* and *enx*. Strain SL150 was a derivative of *Salmonella typhimurium* LT2, whose H antigens had been changed by two successive transductions with a P22 lysate of *S. abony* to the form $b \leftrightarrow enx$ and which was streptomycin- and azide-resistant. It had been prepared 3 years earlier and the subculture now used had been stored on Dorset egg for one year at room temperature in the dark. A number of swarms developed from it, which were at first taken to be either contaminants or mutants having modified *b* or *enx* antigens. However, further investigations showed them to have the antigenic formula: H; $i \leftrightarrow enx$, 0; 4, 5, 12, and to be streptomycin- and azide-resistant. No such organism had ever been known in the laboratory and as the possibility of contamination was ruled out, they were taken to be reversions to the original 1st phase H antigen. At that time the *i* variants were in a very small minority and could only be detected by serum selection from a mass culture from the Dorset egg slope. All single colonies tested had the expected H antigens $b \leftrightarrow enx$ and did not give rise to *i* forms on serum selection. Cultures of SL113, SL141 and SL147, from which SL150 had been derived, were examined in the same way. A small proportion of $i \leftrightarrow enx$ forms was detected in SL141 and SL147, those from the former being resistant to neither streptomycin nor azide, and those from the latter being resistant to streptomycin, but not azide, as expected; SL113 remained entirely $b \leftrightarrow 1.2$. Single colonies from SL141, 147 and 150, all with H antigens $b \leftrightarrow enx$, were stored on Dorset egg slopes at room temperature and at 4°, to see whether they would subsequently develop *i* forms.

In November 1956 a similar series of transductions and selections was made starting from *Salmonella typhimurium* LT2, and were labelled SC113, SC141, SC147 and SC150. These cultures differed from the SL series in being lysogenic for phage P22. No *i* forms could be demonstrated in any of these, and they too were stored on Dorset egg slopes at room temperature and at 4°. The SL and SC series of cultures were examined at intervals for 6 months and no changes were seen.

In June 1957 a detailed study of all available cultures of SL113, SL141, SL150 was begun. These consisted of: (a) the Dorset egg slopes in which the *i* variant had first been found; (b) a duplicate set of Dorset egg slopes kept by Dr Stocker at the Lister Institute; (c) a set of subcultures held by Dr E. S. Anderson of the Central Enteric Reference Laboratory since 1956 when he had phage-typed them; (d) the single colony subcultures kept at room temperature and at 4° since the previous year; (e) a clone from a single cell of strain SL150, isolated by micromanipulation by Dr Quadling at the Lister Institute, in January 1955, and kept on Dorset egg at 4° ever since. In none of these cultures were any organisms found carrying the *b* antigen. Platings direct from the slopes were invariably in the 2nd phase. The 1st phase was isolated by serum selection in Craigie tubes but, as a rule, only after more than 24 hr. incubation; it was in all cases found to be *i*. The second phase was unchanged, as were the O antigens, phage type and streptomycin and azide resistance.

In addition, seven strains of SL150 made colicinogenic by Dr Hamon in November 1956 at the Lister Institute were examined. Three of these had reverted *in toto* in the second phase and four in the first, but none had reverted in both. All were streptomycin-resistant but in some the azide resistance had declined.

At the same time the SC series of cultures was examined. No change in antigenic or other characters was found.

Lysates of members of the SL series prepared at various dates by Dr Stocker and by ourselves were tested for their ability to transduce H antigens and motility to other salmonellas of the same O group. It was found that all the lysates (see Table 2) could transduce motility to SW541, a non-motile strain of *Salmonella typhimurium*, and to SW543, a non-motile variant of *S. paratyphi B* in which the gene determining absence of flagella is closely linked to that controlling the phase I antigen (Stocker, Zinder & Lederberg 1953). Lysates of the unreverted strains failed to transduce the Phase I antigen to *S. typhimurium* LT 2 or to *S. heidelberg* but lysates made after reversion to antigen *i* would transduce that antigen both to *S. heidelberg* and to strain SW543. As it was known that the recipient strains, *S. typhimurium* LT2 and *S. heidelberg*, were in a state in which transductions of antigens in either phase would be manifest (Lederberg & Iino 1956) it can be concluded that the lysates of unreverted strains could not transduce the first phase antigen. However, as it was not known which phase the unreverted strains were in at the time of lysis, no conclusions can be drawn as to the transducibility of the second phase antigen. Lysates of strains which have reverted in the second phase have not yet been tested.

Table 2. *Phage 22 lysates tested for ability to transduce 1st phase antigens to Salmonella heidelberg or S. typhimurium LT2. All these lysates transduced motility to SW541 and SW543*

Strain lysed	Ref. no.	Date prepared	Titre in pfu*/ml.	Transduction of Phase I H antigen
SL113	G27	30 Nov. 53	3×10^9	Nil
SL141	G68	21 Jan. 54	5×10^9	Nil
SL147	G73	2 Feb. 54	5×10^9	Nil
SL150	G86	2 Mar. 54	2×10^8	Nil
SL150	G87	2 Mar. 54	1×10^8	Nil
SL150	G137	9 Dec. 54	2×10^9	Nil
SL150	C1	Nov. 55	5×10^9	Nil
SL150†	C2	July 57	5×10^9	+
SD7	C3	May 58	2×10^9	+
SD13	C4	May 58	1×10^{10}	+
SD14	C5	May 58	1×10^{10}	+

* pfu, plaque forming units.

† Made after reversion of 1st phase antigen.

In August 1957 a third series of cultures was prepared from strain LT2 by transduction with a new P22 lysate of strain SW803. Again no attempt was made to pick non-lysogenic organisms and the first of the series, *b* ↔ 1, 2,

named SD113, carried phage P22. When SD113 was treated with the same lysate, swarms developed whose centres were clear and which were found to have the H antigens $b \leftrightarrow enx$ and to be non-lysogenic. One swarm was subcultured and labelled SD141. Strain SD147 was a streptomycin-resistant variant of SD141. The antigens of this series were not re-examined until October 1957 when it was found that the lysogenic SD113 remained $b \leftrightarrow 1.2$ but both SD141 and 147, which were non-lysogenic, had become entirely $i \leftrightarrow enx$, their O antigens and streptomycin resistance being unaltered. The reverted form of SD147 was altered by transduction to the form $b \leftrightarrow enx$ using the original lysate of *Salmonella abony* (SW 808). This culture is lysogenic and has remained stable.

As all non-lysogenic strains of these three series had reverted to antigen i and none of the lysogenic strains had done so, it seemed that these two factors were associated and an attempt was made to build up another non-lysogenic series to study the process in more detail.

Strain SL270, a double mutant of LT2 requiring tryptophan and methionine, was used as a starting-point to provide extra markers for the recognition of contaminants. Some difficulty was encountered in making non-lysogenic stocks but two sets of lysogenic analogues of SL113 and SL141 were prepared between December 1957 and February 1958. These were designated SD 3, 4, 5 and 6, as set out in Table 3.

In March–May 1958 non-lysogenic analogues of SL113 and 141 were prepared, designated SD 7, 13 and 14, the two latter corresponding to SL141, and the former to SL113. No reversions have so far been observed in either the lysogenic or non-lysogenic stocks stored on Dorset egg slopes for 3 months.

P22 lysates of SD7, 13 and 14 differ from lysates of the original reverting SL series in being able to transduce both b and enx antigens to LT2.

We have tested 22 other non-lysogenic strains of *Salmonella* (obtained from Dr Stocker and from Dr J. Lederberg of Wisconsin) whose H antigens had been altered by transduction and which had been stored on Dorset egg at room temperature, and in none have we found any sign of antigenic instability.

A non-lysogenic strain of *Salmonella heidelberg* (SW 715) prepared in November 1957, using the original lysate of *S. abony* to alter the H antigens to the form $b \leftrightarrow 1.2$, has so far shown no reversions.

In case there should be a natural tendency for a few individual organisms with H antigen i to appear among stocks of *Salmonella* with H antigen b , an investigation was made of old stock cultures of *Salmonella paratyphi B* which had been kept on Dorset egg at room temperature for various lengths of time up to 14 years without subculture, and no i forms could be demonstrated in any of them.

DISCUSSION

The interpretation of the findings reported above is still obscure. Further work is in progress, but so far no method of controlling the occurrence of reversion has been found, and the necessary experiments are consequently slow. It is not known whether all members of a clone eventually revert or

Table 3. Summary of information on the most important strains tested for reversion of transduced antigenic characters

Strain	Date prepared	Parent	Donor		Original H antigens		Reversions			Lyso-genicity for P22
					Ph. I	Ph. II	Date	Ph. I	Ph. II	
SL113	Nov. 53	LT2	SW 803	<i>b-enx</i>	<i>b</i>	1.2	Aug. 57	<i>i</i>	.	-
SL141	Jan. 54	SL113	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	Aug. 56	<i>i</i>	.	-
SL147	Jan. 54	SL141	.	.	<i>b</i>	<i>enx</i>	Aug. 56	<i>i</i>	.	-
SL150	Feb. 54	SL147	.	.	<i>b</i>	<i>enx</i>	Aug. 56	<i>i</i>	.	-
SC113	Oct. 56	LT2	SW 803	<i>b-enx</i>	<i>b</i>	1.2	.	.	.	+
SC141	Oct. 56	SC113	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	+
SC147	Oct. 56	SC141	.	.	<i>b</i>	<i>enx</i>	.	.	.	+
SC150	Nov. 56	SC147	.	.	<i>b</i>	<i>enx</i>	.	.	.	+
SD113	Aug. 57	LT2	SW 803	<i>b-enx</i>	<i>b</i>	1.2	.	.	.	+
SD141	Aug. 57	SD113	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	Oct. 57	<i>i</i>	.	-
SD147	Sept. 57	SD141	.	.	<i>b</i>	<i>enx</i>	Oct. 57	<i>i</i>	.	-
SL341	Nov. 56	SL150	Colicino genic from SW 803	<i>S. sonnei</i> P9	<i>b</i>	<i>enx</i>	Before Aug. 57	.	1.2	-
SL342	Nov. 56	SL150		<i>S. sonnei</i> P9	<i>b</i>	<i>enx</i>		.	1.2	-
SL343	Nov. 56	SL150		<i>E. coli</i> 3609	<i>b</i>	<i>enx</i>		.	1.2	-
SL344	Nov. 56	SL150		<i>E. coli</i> 235	<i>b</i>	<i>enx</i>		<i>i</i>	.	-
SL345	Nov. 56	SL150		<i>E. coli</i> 235	<i>b</i>	<i>enx</i>		<i>i</i>	.	-
SL346	Nov. 56	SL150		<i>E. coli</i> 235	<i>b</i>	<i>enx</i>		<i>i</i>	.	-
SL347	Nov. 56	SL150	<i>E. coli</i> 235	<i>E. coli</i> 235	<i>b</i>	<i>enx</i>	.	<i>i</i>	.	-
SD1	Nov. 57	SW715	SW 803	<i>b-enx</i>	<i>b</i>	1.2	.	.	.	-
SD2	Nov. 57	SD147	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	+
SD3	Nov. 56	SL270	SW 803	<i>b-enx</i>	<i>b</i>	1.2	.	.	.	+
SD4	Dec. 57	SD3	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	+
SD5	Feb. 58	SD270	SW 803	<i>b-enx</i>	<i>b</i>	1.2	.	.	.	+
SD6	Feb. 58	SD5	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	+
SD7	Mar. 58	SL270	SW 803	<i>b-enx</i>	<i>b</i>	1.2	.	.	.	-
SD13	May 58	SD7	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	-
SD14	May 58	SD7	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	-
SD15	May 58	SD7	SW 803	<i>b-enx</i>	<i>b</i>	<i>enx</i>	.	.	.	+

whether only a fraction do so which subsequently outgrow the rest. However, the reported instability is not due to an error in technique or to contamination, and merits some discussion since the main problem of the genetics of transduction is the mechanism by which the transduced fragment is incorporated into the permanent genetic make-up of the recipient.

So far no examples have been published in which transduced characters in *Salmonella* have reverted to the original form and the only analogous case that has been worked out in any detail is that of a series of genes influencing galactose fermentation in *Escherichia coli* K12 (Morse, Lederberg & Lederberg, 1956). It was found in *E. coli* K12 that the transduced *Gal*⁺ genes can persist and be replicated in the host cell along with the original *Gal*⁻ allele of the recipient without being incorporated in the genetic structure of the host. During growth of bacteria there may be crossing over between the transduced fragment ('exogenote') and the main chromosome. Organisms which are partial diploids in this sense are unstable and frequently revert to the haploid state, but it appears that the supernumerary transduced fragment can be reproduced through a long series of cell divisions. Such a situation could account for the present findings if it be postulated that the transduced gene is in some way dominant to the original one which is still present.

The *Gal* system provides an analogy in which the original and transduced characters are reproduced together over many cell divisions. However, the transduced *Gal* character appears to be closely associated with the presence of prophage, while in the present case there is no necessary association of this kind, since the organisms which reverted are non-lysogenic for the transducing phage and fully sensitive to it.

Another important difference between the *Gal* system and the present one is that the lysates of strains carrying transduced *Gal* genes will themselves transduce it, whereas lysates of antigenically unstable strains before reversion do not transduce the Phase I antigenic characters, though they can transduce others. This suggests that there is some chromosomal aberration present. Lysates made after reversion has occurred, transduce the Phase I antigen characters with normal efficiency.

An alternative explanation of reversion to the original antigenic structure is mutation. It might be argued that the original *i* character was part of an essentially stable genotype and that mutative reversions to it would be favoured by natural selection. Some such process may well be responsible for the observed stability of the naturally occurring *Salmonella* types. The main objections to this hypothesis are, first, that no such mutation has been observed in any of the other transduced H antigen characters examined; second, it offers no explanation of the fact that transduced characters in lysogenic strains appear to be stable; and third, that there is no close analogy with mutation from presence of a character to its absence; in the present case the mutation is from synthesis of one protein antigen to another one, antigenically quite different. In addition the independent reversions from *b* to *i* in many separate cultures over a period of eight months indicates a reversion rate higher than would be expected for a mutation.

Neither explanation gives an adequate reason why reversion has so far only been observed at one locus in any one sub-line of SL 141, and not at both, and neither will account for the total disappearance of the non-reverted type.

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