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A DUPLICATION OF THE H_1 (FLAGELLAR ANTIGEN) LOCUS IN SALMONELLA[1]

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TYPICAL Salmonella strains are "diphasic" with respect to their flagellar antigens. That is, they manifest one of two genes, H_1 or H_2, but not both in a given clone until a phase variation takes place and the alternative antigen is expressed.

The genetic analysis of this alternation by transductional methods (LEDERBERG and EDWARDS 1953; LEDERBERG and IINO 1956) has shown that the variation depends on a "change of state" at the H_2 locus. The alternating factor has not been separated by recombination from the determinant for antigenic specificity of H_2. On the other hand, repeated cycles of alternation in state have occurred at H_2 without influencing its antigenic specificity. The H_1 gene, the determinant of the antigen of the alternative phase, is not linked to H_2, but the expression of H_1 depends on the inactive state of H_2. The present paper is part of a comprehensive study of the genetics of Salmonella phase variation (IINO 1958, 1961a,b).

Various anomalies, or exceptions to the simple pattern of diphasic variation have occurred, whose assimilation to the general scheme can throw light on the genic control of protein synthesis. The anomaly presented in this paper is represented by CDC-157, a strain of *Salmonella paratyphi B*, now much used as an antigen for the production of diagnostic anti-*1.2* antiserum (EDWARDS and BRUNER 1942). The advantage of CDC-157 for this purpose is its apparent monophasic behavior; that is, large clones will consist nearly exclusively of cells whose flagellar antigen is *1.2*. A genetic analysis of this strain has revealed two anomalies: (1) The *1.2* antigen is determined by an allele at the H_1 locus instead of the H_2 usual for this antigen, and (2) the stock is carrying a duplicate H_1 locus. The genotypic formula of CDC-157 is thus $H_1{}^bH_1{}^{1,2}$, by contrast to the genotype $H_1{}^bH_2{}^{1,2}$ for typical strains of *S. paratyphi B*. These genotypes accurately summarize the genetic behavior of the respective strains.

MATERIALS, METHODS AND RESULTS

The basic techniques of transductional analysis and background information on Salmonella immunogenetics have been detailed in a previous paper (LEDER-

[1] These studies were mainly conducted at the Department of Genetics, University of Wisconsin in 1953. DR. TETSUO IINO's association with them has done much to clarify the issues brought up here. The work was supported by research grants from the National Cancer Institute, U.S. Public Health Service (C-2157) and the National Science Foundation.

BERG and IINO 1956). To recapitulate, a phage grown on a strain A may carry individual markers, or a small cluster of closely linked markers, to strain B, an operation symbolized A—x B or its equivalent B x—A. The transduced markers replace their homologues in the recipient, an event usually detected by selection against the recipient marker. In this case specific antiserums are used to immobilize cells of the recipient flagellar serotype. The H_1 and H_2 loci are transduced independently, as if unlinked to one another, but H_1 is linked to certain Fla^- (flagellaless, nonmotile) factors. The frequency of transduction is so low, 10^{-5} per marker per phage, that unlinked markers are never, in practice, found together in the same transduction clone.

Strain CDC-157 was kindly furnished by DR. P. R. EDWARDS, of the Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia, and he has also searched his archives for information on its history. A group of some 25 strains was isolated about 1942 from an outbreak of gastroenteritis in the Canal Zone, and identified as *S. paratyphi B*, var. *java*, i.e., monophasic *b* and d-tartrate-positive, phage type 3b. Attempts to select alternative phases by selection with *b*-antiserum met with sporadic success (EDWARDS and BRUNER 1946). CDC-157 itself was such a selection from a culture designated as N25. Further attempts by EDWARDS and BRUNER, and by us to repeat such a selection from this culture, have been unsuccessful, and the two cultures may be accurately designated as monophasic *1.2* and *b* respectively. Another culture, N97, had been isolated from the same outbreak, and also designated as *b*. After it was received from DR. EDWARDS in 1953, however, this culture and single-colony reisolations sporadically generated *1.2* phases after selection in *b* antiserums. The analysis reported here has therefore concentrated on N97 (see Table 1). The sluggishness of phase variation of N97 and its derivatives has undoubtedly been the chief obstacle to this work.

Other strains and techniques have been fully described in previous publications

TABLE 1

History of strains N25, N97 and CDC-157

Original strains from Canal Zone 1942 epidemic	b antiserum selection gave	Then 1:2 antiserum selection gave	Then b antiserum selection gave
N25 b tested <1946	1.2 phase CDC-157	0	. .
N25 b tested 1953	0	. .	. .
N97 b tested 1953	8/8 colonies 1.2 phases	8 tests: 0 2 tests: b phases	0

(STOCKER, ZINDER and LEDERBERG 1953; LEDERBERG and IINO 1956; LEDERBERG 1956). Most of the cultures stem from the type collection in DR. EDWARDS' laboratory (EDWARDS and BRUNER 1942). Many of the specific sera were kindly furnished from the same source and by the Standards Laboratory, Central Public Health Laboratory, Colindale, London. DR. EDWARDS has also confirmed the serotypes of the principal cultures described here.

Variation in strain N97: Upon selection in anti-*b* agar each of eight single colonies of N97 (*b*) gave *1.2* phases, though many of the tubes showed no swarms for several days. But only two of a total of ten isolated *1.2* phases, which may be abbreviated N97 (*b*; *1.2*), would revert to *b* (*b*; *1.2*; *b*). The phases were refractory to further attempts at demonstrating phase variation. Similar trials with N25 (*b*) gave no *1.2* phases, though CDC-157 had been isolated some years before as a stable *1.2* variant from N25 (*b*). The following cultures are therefore available for further study: the original N25 (*b*) and N97 (*b*), presumably of common origin; the N25 (*1.2*) secured in 1946 and N97 (*1.2*) recently obtained from N97 (*b*). At the present time, the N25 cultures are stubbornly monophasic, while variation from *b* $\rightleftarrows$ *1.2* can be demonstrated with some difficulty in N97 cultures.

Transductions with N25 (1.2) = CDC-157: The present study of this strain stems from the findings of transduction experiments that its *1.2* antigen is consistently inherited and at the H_1 rather than at the H_2 locus (Table 2). For example, in transductions from CDC-157 to *S. abony* $H_1{}^bH_2{}^{enx}$ (symbolized CDC-157—x *abony*) the progeny was *1.2:enx*, a serotype which does not occur naturally. This suggests that the *1.2* antigen of CDC-157 is homologous with the *b* ($H_1{}^b$) rather than the *enx* ($H_2{}^{enx}$) antigen of *S. abony*. By contrast, transductions of the *1.2* antigen to *S. abony* from typical diphasic strains such as *S. typhimurium* or *S. paratyphi B* have led to the serotype *b:1.2*, supporting the homology of the typical $H_2{}^{1.2}$ with $H_2{}^{enx}$. These results are different but unambiguous depending entirely on the specific strain used as donor. The other transductions of Table 3 furnish additional support for the formulation of CDC-157

TABLE 2

Transductions from strain CDC-157, presumptive genotype $H_1{}^{1.2}H_2{}^-$

CDC-157 —x:	Genotype H_1	Genotype H_2	Progeny serotype		Inferred genotype H_1	Inferred genotype H_2
a *S. paratyphi* B. SW666	*b*	. .	1.2:	. .	*1.2*	. .
b *S. typhi* H901	*d*	. .	1.2:	. .	*1.2*	. .
c *S. abony*	*b*	*enx*	1.2:	enx	*1.2*	*enx*
d N25 (b)	*b*	. .	1.2:	. .	*1.2*	. .
e *S. miami*	*a*	*1.5*	1.2:	1.5	*1.2*	*1.5*
Compare typical *S. paratyphi B*, strain CDC-3, as donor, presumptive genotype $H_1{}^bH_2{}^{1.2}$						
CDC-3—x:						
cc *S. abony*	*b*	*enx*	b:	1.2	*b*	*1.2*
ee *S. miami*	*a*	*1.5*	b: a:	1.5 1.2	*b* *a*	*1.5* *1.2*

TABLE 3

Transductions to CDC-157 (1.2: –) *as recipient*

CDC-157 x—:	Genotype H_1	Genotype H_2	Progeny serotype		Inferred genotype H_1	Inferred genotype H_2
a. *S. abony*			*b*:	. .	*b*	. .
b.	*b*	*enx*	1.2:	enx	*1.2*	*enx**
c. *S. typhimurium*	*i*	*1.2*	*i*:	. .	*i*	. .
d. *S. paratyphi* B. SW666	*b*	. .	*b*:	. .	*b*	. .

* Further tests were made on this derivative:
3b $H_1^{1.2}H_2^{enx}$ x— *S. typhimurium* $H_1^{i}H_2^{1.2} \rightarrow H_1^{i}H_2^{enx}$ viz., $H_1^{1.2}$ was again replaced by H_1^{i}.

as $H_1^{1.2}$. The results of the preceding sections might be explained on the assumption that H_1^{b} had undergone mutation to $H_1^{1.2}$ rather than phase variation. The recurrence of the change N97 *b* → N97 (*1.2*) cast some doubt on the mutation hypothesis and provoked further study of these strains.

Transduction with N97 (b) and N97 (1.2) as donors (see Table 4): These experiments verify the results already shown with the corresponding strains of N25 and support the assignment of H_1^{b} and $H_1^{1.2}$ to the two phases. N97 therefore displays both H_1^{b} and an unusual $H_1^{1.2}$ in various clones. Two hypotheses might be considered, (1) an anomalous duplication of H_1 in the genotype $H_1^{b}H_1^{1.2}$ or (2) a remarkable rate and direction of mutation at the H_1 locus in a euhaploid: $H_1^{b} \rightleftarrows H_1^{1.2}$. These alternatives could be distinguished by experiments with N97 as recipient.

Transductions to N97 as recipient: These results (Table 5) disqualify the mutation hypothesis and support the duplication of H_1. By a series of single substitutions, N97 (*b*) engenders the following homologous serotypes: *b:1.2* → *b:i* → *b:a* → *c:a*. The last form now carries two standard H_1 alleles from type strain. The mutation hypothesis could not account for the transition of H_1^{b} to $H_1^{1.2}$, H_1^{i}, and H_1^{a} respectively in the first three cases. We may conclude that N97 (*b*) and N97 (*1.2*) are respectively $\boldsymbol{H_1^{b}}H_1^{1.2}$ and $H_1^{b}\boldsymbol{H_1^{1.2}}$.

Table 6 also displays further tests of these genotypes in transductional confrontations. The production of *b:c* as well as *a:c* from H_1^{c}—x $H_1^{a}H_1^{b}$ indicates the equivalence of the two H_1 loci in the recipient.

An H_2 *locus in N97:* No H_2 phase could be elicited from N97 by selection in *b* or *1.2*-antiserum, nor did transductions from N97—x *S. abony* (H_2^{enx}) or *S.*

TABLE 4

Transductions with N97 (b) *and N97* (1.2) *as donor*

N97 (b) —x:	Genotype H_1	Genotype H_2	Progeny serotype		Inferred genotype H_1	Inferred genotype H_2
a *S. typhimurium*	*i*	*1.2*	b:	1.2	*b*	*1.2*
b *S. lomalinda*	*a*	*enx*	b:	enx	*b*	*enx*
c *S. miami*	*a*	*1.5*	b:	1.5	*b*	*1.5*
N97 (1.2)—x						
d *S. abony*	*b*	*enx*	1.2:	enx	*1.2*	*enx*

typhimurium ($H_2^{1.2}$) give any evidence of an H_2 factor in the donor. However, H_2 factors can be transduced in an active form only when the donor strain itself is expressing the H_2 factor (LEDERBERG and IINO 1956). Therefore, it cannot be excluded that N97 (*b*; *1.2*) has the constitution, say, $H_1{}^bH_1{}^{1.2}H_2{}^{1.2}$, the expression of H_2 being subject to an usually sluggish alternation of phase by analogy with $H_1{}^aH_2{}^{enx}$ in *S. abortus-equi* (IINO 1961a).

Explicit evidence for an H_2 locus in N97 comes from the introduction of $H_2{}^{enx}$ by transduction into N97 and its derivatives (Tables 5, 6). Such isolates showed frequent alternation of phase between $H_2{}^{enx}$ and one (only one) of the manifest

TABLE 5

Transductions with N97 (b) *as recipient*

	N97 (b) x—	Genotype H_1	Genotype H_2	Progeny serotype		Inferred genotype H_1	Inferred genotype H_1	Inferred genotype H_2
a				i:	b	*i*	*b*	. .
	S. typhimurium	*i*	*1.2*					
b				i:	1.2	*i*	*1.2*	. .
c				b:	enx	*b*	(*1.2*)	*enx*
	S. abony	*b*	*enx*					
d				1.2:	enx	*1.2*	(*b*)	*enx*

TABLE 6

Transductions with N97 derivatives

	Derivative	—x or x—	Salmonella:	Progeny serotype		Inferred genotype H_1	Inferred genotype H_1	Inferred genotype H_2
a	5a* $H_1{}^iH_1{}^b$	x—	*sendai* $H_1{}^aH_2{}^{1.5}$	a:	b	*a*	*b*	. .
b				b:	c	*b*	*c*	. .
	6a $H_1{}^aH_1{}^b$	x—	*altendorf* $H_1{}^cH_2{}^{1.7}$					
c				a:	c	*a*	*c*	. .
d				a:	enx	*a*	(*c*)	*enx*
	6c $H_1{}^aH_1{}^c$	x—	*abony* $H_1{}^bH_2{}^{enx}$					
e				c:	enx	(*a*)	*c*	*enx*
f				1.2:	enx	*1.2*	(*i*)	*enx*
	5b $H_1{}^iH_1{}^{1.2}$	x—	abony $H_1{}^bH_2{}^{enx}$					
g				b:	1.2	*1.2*	*b*	. .
h	5a $H_1{}^bH_1{}^i$	—x	*miami* $H_1{}^aH_2{}^{1.5}$	i:	1.5	*i*	. .	*1.5*
i				a:	1.2	*a*	. .	*1.2*
	6a $H_1{}^aH_1{}^b$	—x	*typhimurium* $H_1{}^iH_2{}^{1.2}$					
j				b:	1.2	*b*	. .	*1.2*

* Symbols 5a, 6a, etc. refer to the progeny listed in Tables 5 and 6.

H_1 factors. If we assign the genotype $H_1^a H_1^c H_2^{enx}$, the phase variability of H_2^{enx} would be expected to accord with that of its diphasic provenience. However, the control of phase between two H_1 loci, H_1^a and H_1^c is not now understood and is difficult to study when it occurs so rarely in the parental cultures as well as the progeny.

The alternative suggestion must be considered that H_2^{enx} has substituted for an H_1^a to give the diphasic *c:enx*, $H_1^c H_2^{enx}$ (by analogy with the case reported by IINO 1961b). This cannot be disputed except for the strict separation of recognized H_1 and H_2 factors in all other tests reported here. The *c:enx* type was tested —x $H_1^i H_2^{1,2}$ and in the face of some technical problems gave one successful transduction typed as *i:enx* as expected for a regular H_2^{enx} factor.

Are the duplicated factors linked? In a number of technically favorable trials, including several listed here, the H_1 factors of N97 derivatives were inherited independently. Further tests were made —x SW666 Fla_1^- (Table 7) that indicated linkage of each H_1 to Fla^+. However, the Fla^+ might also have been duplicated and we conclude nothing as to the mutual linkage of the duplicated H_1's. Indeed, there is nothing that would firmly contradict a model of complete duplication of the genome, i.e., that N97 is diploid, though H_1 is the only locus known to be heterozygous.

TABLE 7

Linkage of H_1 *factors to* Fla_1

Transductions of N97 derivatives (Table 6) to SW-666

SW-666 Fla_1^- H_1^b x—	Serotypes of Fla^+ selections	Inferred genotypes Fla_1	H_1	H_1
6c $H_1^a H_1^c Fla^+$ in phase a	b: . .	+	*b*	. .
	a: . .	+	a	(*c*?)
6c $H_1^a H_1^c Fla^+$ in phase c	b: . .	+	*b*	. .
	c: .	+	*a*	(*c*?)

Pooled Fla^+ swarms, selected for motility without antiserum, were streaked out, and also selected in b antiserum. The isolated clones were tested and typed as shown.

DISCUSSION

The chief interest of these findings is that an unlikely anomaly still fits the predictions of the genetic model of phase variation with strict separation of H_1 and H_2 homologues. An exception to this rule has been found only by IINO (1961b) who has traced an allele that behaves as H_2^b from an H_1^b parent. It seems most plausible that H_2 originally arose as a duplication of H_1, and still retains some fundamental homology with it, though this cannot usually be demonstrated. Indeed, synaptic homology should depend on the regional quality of a chromosome segment, beyond a single gene (ZINDER 1960).

The phenotypic confusion of $H_1^{1,2}$ and $H_2^{1,2}$ is paralleled by the *lw* antigen. *Salmonella wien* must be typed as $H_1^b H_2^{lw}$, and *S. dar-es-salaam* as $H_1^{lw} H_2^{enz18}$ to interpret the findings of EDWARDS, DAVIS and CHERRY (1955). In contrast to *1.2*, *lw* has long been familiar to Salmonella immunologists as behaving in some

strains as a phase-1, in others as phase-2. The inference is suggested by the pattern of alternative phases and is given genetic meaning by the transduction tests.

SPICER and DATTA (1959) have reported a clone of *S. typhimurium* as giving unstable transductional derivatives which show evidence of diploidy at both the H_1 and H_2 loci. It differs from the present case in part in the irreproducibility of the effect and transduction of the duplicated H_1 locus or loci to other strains.

In typical diphasic strains, we can envisage that the H_1 gene is repressed by the action of the H_2 gene, or perhaps of a variable phase controller immediately adjacent to it. No evidence of a controller at the H_1 locus has been found in typical diphasic H_1H_2 strains, only in the response of H_1 to H_2 control. This system has evidently broken down in the H_1H_1 duplications; but we cannot say whether a residual H_2 controller is still coupled to one H_1 or whether another gene is pinch hitting for the evident regulation.

Apart from the troublesome obstinacy of N97 in undergoing phase variation, genetic analysis by selective transduction has evident disadvantages for the articulation of a complex system. We may look forward to constructive applications of sexual breeding methods in Salmonella immunogenetics (BARON, CAREY and SPILMAN 1959).

SUMMARY

Salmonella paratyphi B strain CDC-157 has the genotype $H_1{}^bH_1{}^{1,2}H_2{}^f$; typical strains of this serotype are $H_1{}^bH_2{}^{1,2}$. Two anomalies are thus evident, the duplication of H_1 and the representation of the *1.2* antigen at the H_1 locus. The transductional progeny of CDC-157 affirm the assigned genotype. They include such exceptional serotypes as *1.2:1.5*, *1.2:enx*, *b:i*, *b:c*, and *a:c*.

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