Bull Org. mond. Santé }
Bull. Wld Hlth Org. } 1964, 30, 845-853

Segregation of Genetic Factors during Recombination in *Vibrio cholerae*, Strain 162

K. BHASKARAN, M.B., B.S., Ph.D.[1]

In this paper an analysis of genetic recombinants derived from crosses between two mutant stocks of Vibrio cholerae, *strain 162, differing from one another in nutritional requirements and other characters, is presented. It is shown that parent strains possessing a fertility factor (designated as the P factor) function as gene-donors while P⁻ strains (without the P factor) serve as gene-recipients. Linkage between two genetic factors (purine and valine + isoleucine) is demonstrated and the probable sequence of seven genetic factors in the chromosome of* V. cholerae, *strain 162, is inferred.*

In a previous paper (Bhaskaran, 1960) it was shown that certain mutant stocks of *Vibrio cholerae* differing in nutritional requirements and other characters, when seeded together in pairs on selective media, yielded colonies which displayed a reassortment (or recombination) of parental characters. This finding was explained on the hypothesis that conjugation between the parent cells occurred and as a result genetic material was transferred from cell to cell, giving rise to hybrids. Such a phenomenon is well known in *Escherichia coli* (Lederberg, 1947; for recent review see Hayes, 1962), and has recently been observed in *Salmonella typhimurium* by Ozeki and Stocker (see Stocker, 1960), and Smith & Stocker (1962). As in these genetic systems, the ability to conjugate, or fertility, in *V. cholerae* is also determined by a fertility factor—designated in this case as the P factor—which should be present in one of the parent cells employed in crosses. Thus recombinants result if P^+ strains (possessing the P factor) are crossed with P^- strains (devoid of the P factor) and not from $P^- \times P^-$ crosses. As P^+ strains also produce clearings or lysis when spotted on P^- strains in semisolid nutrient media, and as this phenomenon is not due to any demonstrable bacteriophage, it may be that the P factor determines the production of a bactericidal agent, the nature of which is obscure. In this respect the genetic system in *S. typhimurium*, referred to above, is very similar, as strains that are colicinogenic, particularly for colicine I, or *col I* strains, yield recombinants when crossed with strains which do not produce this colicine, or *col*⁻ strains. This colicinogenic factor has also been shown to mediate conjugation in *E. coli* (Clowes, 1961).

Studies carried out on *E. coli* and *S. typhimurium* have revealed that strains possessing the fertility factor, such as F^+ strains of *E. coli* (F being the general label for fertility factors in this species) and *col I* strains of *S. typhimurium*, function as gene donors (males) while the corresponding F^- and *col*⁻ strains function as gene acceptors (females). It appears probable that these fertility factors are cytoplasmic units multiplying autonomously in the cell and at a faster rate than the host bacterium, so that they are capable of contagious infection of other cells by conjugation. Genetic recombinants, however, result only when chromosomal segments are also transferred from donor to recipient at the same time, and this in general occurs at a much reduced frequency. In certain variants of F^+ strains of *E. coli* (Hfr strains) it is seen that the F factor is not generally transmissible from cell to cell although the frequency of genetic recombinants is considerably enhanced. This is interpreted as possibly due to integration of the F factor with the host chromosome in these strains, thus restricting the transmission of that factor to other cells. A similar condition (i.e., alternation between a cytoplasmic phase and a nuclear phase) is also known with certain temperate bacteriophages and at least one colicinogenic factor (Alfoldi et al., 1958) and such agents are now

[1] Professor of Microbiology, All India Institute of Hygiene and Public Health, Calcutta, India. Present address: Senior Scientific Officer, Department of Microbiology, Central Drug Research Institute, Lucknow, India.

designated as "episomes" (Jacob & Wollman, 1958), a term which is now extended to include other as yet unidentified factors in various bacteria (Sneath, 1962).

Despite the fact that the P factor of *V. cholerae* is infective and easily transmissible from P^+ to P^- cells in mixed populations, studies carried out so far (Bhaskaran, 1959, 1960) have not shown by genetic data whether genetic migration is also in the same direction, although certain inactivation experiments with ultraviolet rays were very suggestive (Bhaskaran & Iyer, 1961). In these experiments, if the P^+ parent population was exposed to a source of ultraviolet rays to reduce the viable count by a factor of 1/1000 to 1/10 000 and then crossed with normal P^- parents, recombinants appeared; this showed that non-viable P^+ cells were still able to conjugate with P^- cells. If the procedure was reversed, recombination was suppressed, which could only mean that the viability of the P^- parent (or recipient) was essential for the development of recombinant colonies. The analysis of recombinants, by which the roles of the donor and recipient could be confirmed, has suffered from the limitation that the number of markers distinguishing the parent strains are few, particularly those which remain unselected by the technical procedures adopted for the isolation of recombinants, i.e., those characters either allele of which was free to occur in the recombinants.

The present paper furnishes the results of crosses with new mutant stocks of *Vibrio cholerae*, strain 162, with a wider range of distinguishing markers than hitherto employed. The analysis of recombinants isolated from these crosses provides adequate evidence to evaluate the roles of P^+ and P^- strains in the phenomenon, and permits valid inferences regarding linkage effects and the probable sequence of certain genetic factors in the chromosome of *V. cholerae*, strain 162.

MATERIALS AND METHODS

Strains

P^- and P^+ derivatives of V58/str-s, V58/str-r, V63/str-s and V63/str-r, derived from *V. cholerae*, strain 162, were employed in this study. V58 differed from V63 in nutritional requirements and O antigenic type as shown below:

V58 = pur^+ $ilva^-$ O-Og arg^- leu^+ his^-
V63 = pur^- $ilva^+$ O-In arg^+ leu^- his^+

Key: pur = purine (guanosine)
ilva = valine+isoleucine
arg = arginine
leu = leucine
his = histidine
(+) = indicates independence
(−) = indicates dependence
O-Og = O antigenic type Ogawa
O-In = O antigenic type Inaba
str-s = streptomycin-sensitive
str-r = resistant to streptomycin (500 μg/ml)

Media

Nutrient broth, nutrient agar and sloppy nutrient agar were prepared as described earlier (Bhaskaran, 1958). The basal minimal medium employed was as described earlier (Bhaskaran & Rowley, 1956) supplemented with L-asparagine (0.01% w/v) and DL-methionine (0.01%w/v).

Isolation of recombinants

20 ml of nutrient broth contained in 100-ml Erlenmeyer flasks were seeded from overnight agar slant cultures of the strains individually. After 3 hours' incubation at 37°C without aeration, the cultures were centrifuged at 2500 r.p.m. for 10 minutes. The deposits were suspended in 10 ml minimal medium and centrifuged again. The bacterial deposits were then resuspended in 1 ml minimal medium and employed in recombination tests. 0.05 ml of each parent culture was mixed and spread on the surface of selective minimal agar for the isolation of recombinants. The plates were incubated at 37°C for 72 hours and the colonies which appeared were investigated further.

Study of recombinants

Colonies which appeared on recombination plates were cultivated again on the same medium as that on which they were isolated. The precise nutritional requirements of each recombinant was then determined by seeding on minimal media containing any two of the three nutritional factors present in the selective medium. Thus, for example, every colony which appeared on pur arg his minimal agar was subsequently tested for growth on pur arg, arg his and pur his minimal agar. From the growth pattern on these media the nutritional requirement of each recombinant could be determined.

O antigenic type determinations and streptomycin-sensitivity tests were carried out by spotting each colony on nutrient agar and nutrient agar containing streptomycin (500 μg/ml). The former

was subjected to the sloppy agar technique of antigenic type determination described previously (Bhaskaran & Gorrill, 1957), while the occurrence of growth on streptomycin nutrient agar indicated resistance to the antibiotic.

RESULTS

As a rule, crosses were carried out between V63 (pur^- $ilva^+$ arg^+ leu^- his^+) and V58 (pur^+ $ilva^-$ arg^- leu^+ his^-) sublines. Four crosses were possible by alternating streptomycin-resistance (str-r) and the P factor in the parent strains as follows:

V58/str-s P^+ × V63/str-r P^- (cross IA)
V58/str-r P^+ × V63/str-s P^- (cross IB)
V58/str-r P^- × V63/str-s P^+ (cross 2A)
V58/str-s P^- × V63/str-r P^+ (cross 2B)

As the parent strains differed from one another in five nutritional markers, recombination between these factors would give rise to colonies on minimal media inadequate for the growth of either strain. In order to secure all such recombinants, it was of advantage to use minimal media containing either pur or leu (required by V63) and any two of the three nutritional factors ilva, arg and his (required by V58). Six selective combinations of the nutritional factors were possible, and each such combination was employed for the isolation of recombinants. Unsupplemented minimal agar was not employed, as previous trials had shown that prototrophic recombinants (pur^+ $ilva^+$ arg^+ leu^+ his^+) were of rare occurrence in all crosses.

In these experiments there was every possibility that reverse mutations in the parent strains, such as pur or leu independence in V63 and ilva or arg or his independence in V58, might occur and might be scored falsely as recombinants. However, in controls employing sterile P^- × P^- crosses of the parents, the numbers of colonies which appeared on the same media as those employed for the isolation of recombinants from P^+ × P^- crosses were very few. This justified the use of such complex media for the isolation of recombinants. As an additional precaution, P^- × P^- crosses were put up as controls every time P^+ × P^- crosses were carried out.

Table 1 gives details of the frequency of occurrence of the more common recombinant classes derived from cross 1 (A & B) and cross 2 (A & B). For each of these crosses it will be seen that the results are similar in A and B, which shows that the alternation of streptomycin-resistance between the parents influences in no way the pattern of recombinants. However, recombinants derived from cross 1 are quite different from those derived from cross 2, even when the same selective markers of the parents were employed. This deviation in the frequency of various recombinant classes in the two crosses is only explicable by the reversal of the P factor in the parent strains.

The genotype of the various recombinant classes generally comformed to those of the P^- parent, the extraneous allele in most cases being the selective marker of the P^+ parent. An exception to this general finding so far is the occurrence of recombinants with the genotype pur^+ $ilva^-$ arg^+ leu^- his^+ in cross 1 that display two genetic factors derived from the P^+ parent, only one of which (pur^+) is the selective marker of the P^+ parent in this cross. This is indicative of linkage of pur and ilva loci in strain V58 and this linkage is seen in V63 as well by the occurrence of pur^- $ilva^+$ arg^- leu^+ his^- recombinants in cross 2.

These results clearly indicate that in each recombinant class the genetic contributions of the two parent strains are unequal, the P^- parent bearing the major share. Such a phenomenon could not be expected if the initial zygote formed by conjugation between the parent cells, prior to the segregation of recombinants, was a complete diploid heterozygote, as the frequency of various recombinant classes would then be similar in both crosses. As in the case of *E. coli* and *S. typhimurium*, it appears very likely that the P^+ parent functions as the donor (male) cell, contributing only chromosomal fragments to the P^- parent which functions as the recipient (female).

With this general evidence pointing to the role of P^+ and P^-, as donor and recipient, during genetic recombination in *Vibrio cholerae* it was logical to investigate whether a complete analysis (with respect to selective and unselective markers) of the frequent as well as the infrequent classes of recombinants would permit any inference regarding the sequential order of the various genetic factors.

Table 2 provides such a study of 730 recombinants isolated from cross 1 ($V58P^+$ × $V63P^-$). The genotype of the recombinants is indicated in a manner which, on inspection, would reveal what alleles of the P^+ parent are present in each recombinant class, and linkage between genetic factors could be inferred by the frequency of their occurrence. In this cross the most frequent recombinant classes are those in which the P^+ parent contributes either the leu^+ or pur^+ factor alone, accounting for 374 and

TABLE 1

EFFECT OF REVERSAL OF P POLARITY ON GENETIC RECOMBINATION IN *VIBRIO CHOLERAE* [a]

Recombinant class	Cross 1				Cross 2			
	Selective marker		P^+ parent = pur^+ $ilva^-$ arg^- leu^+ his^- P^- parent = pur^- $ilva^+$ arg^+ leu^- his^+		Selective marker		P^+ parent = pur^- $ilva^+$ arg^+ leu^- his^+ P^- parent = pur^+ $ilva^-$ arg^- leu^+ his^-	
	P^+	P^-	1A [b]	1B [b]	P^+	P^-	2A [b]	2B [b]
pur^+ $ilva^+$ arg^+ leu^- his^+	pur^+	his^+	16/46	17/44	his^+	pur^+	3/50 [c]	3/38 [c]
pur^+ $ilva^-$ arg^+ leu^- his^+			30/46	23/44			0/50	0/38
pur^+ $ilva^-$ arg^- leu^+ his^+			0/46	4/44 [c]			47/50	35/38
pur^+ $ilva^+$ arg^+ leu^- his^+	pur^+	arg^+	33/49	69/99	arg^+	pur^+	3/50 [c]	1/49 [c]
pur^+ $ilva^-$ arg^+ leu^- his^+			12/49	30/99			0/50	0/49
pur^+ $ilva^-$ arg^+ leu^+ his^-			3/49 [c]	0/99			47/50	44/49
pur^+ $ilva^+$ arg^+ leu^- his^+	pur^+	$ilva^+$	41/41	64/65	$ilva^+$	pur^+	5/50 [c]	6/39 [c]
pur^+ $ilva^+$ arg^- leu^+ his^-			0/41	1/65 [c]			45/50	33/39
pur^- $ilva^+$ arg^+ leu^+ his^+	leu^+	his^+	52/60	38/56	his^+	leu^+	0/60	2/88 [c]
pur^+ $ilva^-$ arg^- leu^+ his^+			6/60 [c]	15/56 [c]			59/60	86/88
pur^- $ilva^+$ arg^+ leu^+ his^+	leu^+	arg^+	57/60	88/90	arg^+	leu^+	2/59 [c]	0/100
pur^+ $ilva^-$ arg^+ leu^+ his^-			3/60 [c]	1/90 [c]			57/59	100/100
pur^- $ilva^+$ arg^+ leu^+ his^+	leu^+	$ilva^+$	54/57	85/99	$ilva^+$	leu^+	3/60 [c]	2/90 [c]
pur^+ $ilva^+$ arg^- leu^+ his^-			2/57 [c]	1/99 [c]			24/60	45/90
pur^- $ilva^+$ arg^- leu^+ his^-			0/57	0/99			33/60	43/90

[a] The frequency of each recombinant class is indicated as a fraction, the numerator representing the number of colonies belonging to the class and the denominator the total number of colonies tested from the selective medium.

[b] In crosses under A the P^+ parent is streptomycin-sensitive and the P^- parent streptomycin-resistant. In B these characters are reversed between the parent strains.

[c] Probably represent reverse mutations in the parent P^+ strains.

TABLE 2. ANALYSIS OF RECOMBINANTS FROM CROSSES BETWEEN *V. CHOLERAE* STRAINS [a] V58 P$^+$ AND V 63P$^-$

Recombinant class [b]	Selective marker of donor	Selective marker of recipient						Total
		arg$^+$		his$^+$		ilva$^+$		
		Cross A [c]	Cross B [c]	Cross A [c]	Cross B [c]	Cross A [c]	Cross B [c]	
	leu$^+$							
leu$^+$		57	88	52	38	54	85	374
arg$^-$ leu$^+$		X [d]	X [d]	2	2	1	11	16
leu$^+$ his$^-$		0	0	X [d]	X [d]	0	1	1
pur$^+$ ... leu$^+$		0	1	0	0	0	1	2
str$_i$ pur$^+$ ... O-Og arg$^-$ leu$^+$		X [d]	X [d]	0	1	0	0	1
	pur$^+$							
pur$^+$		33	68	15	14	40	60	230
pur$^+$ ilva$^-$		11	28	24	19	X [d]	X [d]	82
str$_i$ pur$^+$ ilva$^-$		1	1	2	1	X [d]	X [d]	5
str$_i$ pur$^+$		0	1	1	3	1	1	7
pur$^+$ ilva$^-$ O-Og		0	1	3	1	X [d]	X [d]	5
pur$^+$ ... leu$^+$		1	0	0	0	0	0	1
str$_i$ pur$^+$ ilva$^-$ O-Og		0	0	1	2	X [d]	X [d]	3
pur$^+$ ... O-Og		0	0	0	0	0	3	3
Total								730

[a] Donor (V58 P$^+$) : str$_i$ pur$^+$ ilva$^-$ O-Og arg$^-$ leu$^+$ his$^-$. Recipient (V63 P$^-$) : str$_o$ pur$^-$ ilva$^+$ O-In arg$^+$ leu$^-$ his$^+$.

[b] In each recombinant class only allele(s) derived from the donor is (are) shown, the other alleles being those of the recipient.

[c] In crosses under A the P$^+$ parent is streptomycin-sensitive and the P$^-$ parent streptomycin-resistant. In B these characters are reversed between the parents. Str$_i$ indicates the streptomycin allele of the donor (P$^+$) strain, while str$_o$ indicates that of the recipient (P$^-$) strain.

[d] Classes which could not have been detected on the selective medium used.

230 recombinants respectively. Next in frequency are recombinants which receive the segment pur$^+$ ilva$^-$ from the P$^+$ parent (82 colonies). This, as pointed out earlier, is evidence of close linkage between pur and ilva. As all other recombinant classes are few in number, such close linkage is not evident between other genetic factors. However, it will be seen that:

(*a*) arg$^-$ segregates with leu$^+$ (16 colonies),

(*b*) str$_i$ segregates with pur$^+$ (16 colonies), and

(*c*) O-Og segregates with pur$^+$ ilva$^-$ (8 colonies).

Except for one recombinant in which his$^-$ segregates with leu$^+$, his$^-$ appears to be independent of the rest.

Results recorded in Table 3 (cross V63P$^+$ $\times$ V58P$^-$) show that ilva$^+$, his$^+$ and arg$^+$ segregate

TABLE 3

ANALYSIS OF RECOMBINANTS DERIVED FROM CROSSES BETWEEN *V. CHOLERAE* STRAINS [a] V63 P+ AND V58 P−

Recombinant class [b]	Selective marker of donor	Selective marker of recipient				Total
		pur+		leu+		
		Cross A [c]	Cross B [c]	Cross A [c]	Cross B [c]	
	ilva+					
ilva+		41	31	23	42	137
pur− ilva+		X [d]	X [d]	24	37	61
pur− ilva+ O-In		X [d]	X [d]	8	6	14
str_i pur− ilva+		X [d]	X [d]	1	0	1
ilva+ O-In		4	2	1	2	9
str_i ... ilva+		0	0	0	1	1
str_i ... ilva+ ... arg+ leu− his+		1	0	X [d]	X [d]	1
	his+					
his+		47	35	58	86	226
O-In ... his+		0	0	1	0	1
arg+ ... his+		0	0	1	0	1
	arg+					
arg+		46	44	56	97	243
O-In arg+		1	0	1	3	5
arg+ leu−		0	4	X [d]	X [d]	4
Total						704

[a] Donor (V63 P+) : str_i pur− ilva+ O-In arg+ leu− his+. Recipient (V58 P−) : str_o pur+ ilva− O-Og arg− leu+ his−.

[b] In each recombinant class only the allele(s) derived from the donor is (are) shown, the other alleles being those of the recipient.

[c] In crosses under A the P+ parent is streptomycin-sensitive and the P− parent streptomycin-resistant. In B these characters are reversed between the parents. Str_i indicates the streptomycin allele of the donor (P+) strain while str_o indicates that of the recipient (P−) strain.

[d] Classes which could not have been detected on the selective medium used.

independently, accounting for 137, 226 and 243 colonies respectively, and there is no evidence of close linkage between them. Linkage between pur and ilva is confirmed in strain V63P+ also (76 colonies). The other significant findings in this cross are:

(*a*) segregation of O-In with ilva+ (23 colonies), and

(*b*) segregation of O-In with arg+ (5 colonies).

As in cross 1, leu− segregates with arg+ (4 colonies).

Pooling the data of Tables 2 and 3, one may postulate that the sequential order of various genetic factors in these strains as

str ... pur ilva ... O ... arg ... leu ... his,

in a single linkage group.

Such a gene order accounts for 1423 out of 1434 recombinants isolated from crosses 1 and 2 as resulting from recombination between a single chromosomal fragment of the P^+ parent with the chromosome of the P^- parent by either one or two cross-overs. The remaining 11 colonies (7 in cross 1 and 4 in cross 2, these recombinants being indicated in Tables 2 and 3 by a discontinuity of the genetic factors derived from the P^+ parent) probably represent either recombination of more than one chromosomal fragment of the P^+ parent with the P^- chromosome or multiple cross-overs between them.

The analysis of recombinants in Tables 2 and 3 further reveals that in a large proportion of the recombinants (1210 out of 1423 colonies) the contribution of the P^+ parent is restricted to single genetic factors and, except in the case of the linked factors pur and ilva which appear in 143 recombinants, contribution of more than one genetic factor appears to be infrequent. This may indicate that during conjugation in *V. cholerae* the chromosomal fragments transmitted from cell to cell are generally small and comparable to single genetic factors transduced by bacteriophages (Zinder & Lederberg, 1952). On rare occasions, however, the segment seems to be large enough to accommodate at least four genetic factors, of which only two are closely linked (segment str_i pur^+ $ilva^-$ O-Og; see Table 2).

The rarity of such recombinants, unlike those in the *E. coli* and *S. typhimurium* genetic systems referred to earlier, is perhaps due to rupture of the migrating segment occurring far more frequently owing to the extraordinary motility of vibrio strains which would tend to interrupt the matings frequently. Comparative studies with non-motile mutants of the parent strains would show whether there is any relationship between motility and chromosomal recombination in this genetic system.

DISCUSSION

The findings recorded in this paper support what appears to be essentially the basic theme of genetic recombination in bacterial species resulting from conjugation and mediated by fertility factors. In this study, as in similar though more detailed studies on *E. coli* and *S. typhimurium*, the analysis of recombinants points to one parent functioning as a gene-donor while the other parent serves as a recipient. With the data available it has also been possible to map the probable order of at least seven genetic factors in the chromosome of *V. cholerae*, strain 162, of which two (pur and ilva) are closely linked.

That the donor ability is associated with P^+ strains of *V. cholerae* is to be expected, as the P (fertility) factor itself is easily transmitted to P^- cells in mixed populations and, as in $F^+ \times F^-$ crosses in *E. coli* and *col* $I \times col^-$ crosses in *S. typhimurium*, all recombinants so far tested have proved to be P^+ (Bhaskaran, 1960).

This genetic system in *V. cholerae* is in many respects analogous to genetic recombination in *S. typhimurium* (Smith & Stocker, 1962) and *E. coli* (Clowes, 1961) mediated by colicinogenic factors. Although P^+ strains of *V. cholerae* do produce a bacteriocine of some sort, conventional methods employed for their detection, as for colicinogenic strains of *E. coli*—for example, exposing sensitive strains to colicines present in chloroform-killed cultures of colicinogenic strains—do not demonstrate the lytic effect of P^+ on P^- strains. The best way to show this is by the technique generally favoured in bacteriophage work (Adams, 1960) in which the indicator is seeded in a lawn of semisolid agar, on the surface of which drops of lysogenic cultures are placed. It is tempting to speculate whether the clearings produced by P^+ on P^- strains when tested in this way are the result of the partial anaerobic condition present in the semisolid layer in which the lytic agent is probably well-developed, as reported in the case of a lytic factor observed in *V. cholerae* produced under anaerobic conditions (Farkas-Himsley & Seyfried, 1962).

Although the lytic phenomenon is exhibited by viable P^+ cells spotted on a lawn of a P^- culture, viability of the P^+ cells is not essential for the transfer of this character to P^- cells. It was seen, while working on the effect of ultraviolet irradiation on P^+ and P^- cultures in mating mixtures referred to earlier (Bhaskaran & Iyer, 1961), that inactivated P^+ cells were still capable of transmitting the P factor to P^- cells. This was demonstrated by determining the viable count of P^+ cultures after irradiation, using the conventional dilution and plating technique and estimating at the same time the number of infectious centres (clearings) produced by these cultures by seeding aliquots of various

dilutions along with P^- cells in semisolid agar. In such parallel tests it was seen that the number of infectious centres was approximately one hundred times greater than would be expected if only viable cells produced the lytic agent or transmitted this ability to other cells. When tests were made on the growth from the infectious centres produced by inactivated cells (from those dilutions not revealing any viable cells), no cells of the irradiated parent could be isolated, and the growth was a pure culture of P^+ derivatives of the original P^- strain. Thus the lytic phenomenon could be explained only as the effect of a clone of P^+ cells arising by infection with the P factor from non-viable P^+ cells.

Stocker, Smith & Ozeki (1963) report that certain colicinogenic factors may be lost in some strains through keeping the cultures at room temperature for some weeks, and a similar instability of the P factor in *V. cholerae* was reported earlier (Bhaskaran, 1960). Iyer (personal communication) has shown that if broth cultures of P^+ strains kept at 37°C are plated out at weekly intervals, more than 90% of the colonies isolated from the cultures after five weeks were P^-. This instability may indicate that the P factor is purely a cytoplasmic constituent not integrating with the host chromosome, which would tend to stabilize the factor in the cell. In this connexion Clowes (1963) has shown, with experimental evidence, that colicinogenic or C factors are best regarded as cytoplasmic units (plasmids) rather than as episomes.

The methods adopted with *E. coli* for demonstrating the unidirectional transfer of genetic material have not proved applicable to *V. cholerae*. Hayes (1952) observed that F^+ cells sterilized with streptomycin could be successfully crossed with viable F^- cells, while the reverse procedure rendered the cross sterile. The conclusion drawn was that the presumptive zygote was formed in F^- cells which should remain viable for recombinants to develop. Such tests in *V. cholerae* showed that the recombination rate was considerably reduced if P^+ cells were treated with streptomycin, or if the test was done on streptomycin-containing media to which the P^+ culture alone was sensitive, so that no valid conclusions could be drawn. A direct demonstration of transfer of genetic material from cell to cell in *E. coli* was later possible when variants of F^+ (Hfr variants) were crossed with F^- cells which yielded a high frequency of recombinants. In such crosses it was shown by interrupted mating experiments that the length of segments transferred from Hfr to F^- cells was up to a limit proportional to the period of conjugation. In *V. cholerae* such studies have not so far been possible.

In *E. coli* and *S. typhimurium* the chromosome is regarded as circular, from data that reveal linkage of genetic factors earlier considered to be at the distal ends of linear segments. With the few markers available for analysis in *V. cholerae*, no definite conclusions can be drawn at present on the nature of the chromosome in *V. cholerae*, strain 162.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Indian Council of Medical Research. The author is indebted to Dr B. A. D. Stocker, Lister Institute of Preventive Medicine, London, for his comments on the manuscript and suggestions regarding the presentation of the genetic data. The assistance of Mr Jagadish Majumdar is acknowledged.

RÉSUMÉ

On sait que certaines souches de *Vibrio cholerae* peuvent être croisées entre elles et que les souches qui en sont issues présentent une recombinaison des caractères parentaux. On en a conclu à une conjugaison entre souches parentes et à un transfert génétique. Comme dans d'autres systèmes génétiques — ceux de *Escherichia coli* et de *Salmonella typhimurium* — un facteur de fertilité, qui doit exister chez l'un ou l'autre parent est indispensable à la conjugaison. Chez *V. cholerae*, la nature de ce facteur « P » est inconnue; il est probable que c'est une bactériocine.

Dans le présent article, l'auteur expose les résultats d'une étude portant sur la souche 162 de *V. cholerae*, ayant une gamme de marqueurs plus étendue que les souches étudiées jusqu'ici. Ces recherches avaient pour but *a*) d'évaluer le rôle respectif de chacune des souches parentales et de déterminer celle qui possédait le facteur P (souche P^+); *b*) de préciser l'ordre de séquence des fac-

teurs génétiques dans les chromosomes de *V. cholerae* souche 162.

Les mutants employés dans cette étude étaient les suivants: 1) V63, appartenant au type O Inaba, caractérisé par sa dépendance envers la purine et la leucine (pur^-, leu^-) et son indépendance vis-à-vis de l'isoleucine-valine ($ilva^+$), l'histidine et l'arginine (his^+, arg^+); 2) V58, appartenant au type O Ogawa, caractérisé par sa dépendance envers l'isoleucine-valine ($ilva^-$), l'histidine et l'arginine (his^-, arg^-), et son indépendance à l'égard de la purine (pur^+) et de la leucine (leu^+). Des dérivés de chacune de ces souches, résistants à la streptomycine, ont aussi été employés.

Il a été possible d'effectuer quatre croisements entre un parent sensible à la streptomycine et le dérivé résistant de l'autre, en interchangeant le facteur P entre eux. Les produits de la combinaison ont été isolés sur des milieux sélectifs; les facteurs nutritionnels ont été utilisés comme marqueurs dans toutes sortes de combinaisons.

Les résultats de ces croisements ont montré que le génotype des hybrides est en général celui du parent P^-, l'allèle étranger étant en général le marqueur sélectif du parent P^+. Cette constatation vient à l'appui de l'idée que le parent P^+ fonctionne comme gène-donneur, ne cédant en général qu'une infime partie de son chromosome au parent P^- qui joue le rôle de gène-récepteur. Le linkage entre les caractères « pur » et « ilva » a été démontré expérimentalement.

Il a été possible de préciser comme suit la séquence des facteurs génétiques: str (pur ilva) O arg leu his. Cela étant admis, on a pu expliquer que 1423 de 1434 hybrides examinés résultent de la combinaison entre un fragment chromosomique du parent P^+ avec le chromosome du parent P^-, par un ou deux crossing over.

REFERENCES

Adams, M. H. (1950) *Methods of study of bacterial viruses*. In: Comroe, J. H., ed., *Methods in medical research*, Chicago, The Year Book Publishers, vol. 2, p. 3

Alfoldi, L., Jacob, F., Wollman, E. L. & Maze, R. (1958) *C.R. Acad. Sci. (Paris)*, **246**, 3531

Bhaskaran, K. (1958) *J. gen. Microbiol.*, **19**, 71

Bhaskaran, K. (1959) *Indian J. med. Res.*, **47**, 253

Bhaskaran, K. (1960) *J. gen. Microbiol.*, **23**, 47

Bhaskaran, K. & Gorrill, R. H. (1957) *J. gen. Microbiol.*, **16**, 721

Bhaskaran, K. & Iyer, S.S. (1961) *Nature (Lond.)*, **189**, 1030

Bhaskaran, K., & Rowley, D. (1956) *J. gen. Microbiol.*, **15**, 417

Clowes, R. C. (1961) *Nature (Lond.)*, **190**, 988

Clowes, R. C. (1963) *Genet. Res.*, **4**, 162

Farkas-Himsley, H. & Seyfried, P. L. (1962) *Nature (Lond.)*, **193**, 1193

Hayes, W. (1952) *Nature (Lond.)*, **169**, 118

Hayes, W. (1962) *Brit. med. Bull.*, **18**, 36

Jacob, F. & Wollman, E. L. (1958) *C. R. Acad. Sci. (Paris)*, **247**, 154

Lederberg, J. (1947) *Genetics*, **32**, 505

Smith, S. M. & Stocker, B. A. D. (1962) *Brit. med. Bull.*, **18**, 46

Sneath, P. H. A. (1962) *Brit. med. Bull.*, **18**, 41

Stocker, B. A. D. (1960) *Introduction : microorganisms in genetics*. In: Hayes, W. & Clowes, R. C., ed., *Microbial genetics. (Tenth symposium of the Society for General Microbiology, London, April 1960)*, Cambridge, University Press, p. 1

Stocker, B. A. D., Smith, S. M. & Ozeki, H. (1963) *J. gen. Microbiol.*, **30**, 201

Zinder, N. D. & Lederberg J. (1952) *J. Bact.*, **64**, 679