Mapping of a gene causing resistance to chlorate in *Salmonella typhimurium*

A. H. STOUTHAMER AND CORRIE W. BETTENHAUSSEN

Microbiology Department, Biological Laboratory, Free University, De Boelelaan 1087, Amsterdam, The Netherlands

STOUTHAMER, A. H. and BETTENHAUSSEN, C. W. 1970. Mapping of a gene causing resistance to chlorate in *Salmonella typhimurium*. Antonie van Leeuwenhoek **36**: 555-565.

Chlorate-resistant mutants of *S. typhimurium* LT2 and LT7 and of *S. abony* have been isolated, which are deficient in the biosynthesis of nicotinic acid and thiamin and in the fermentation of inositol. These mutants could be divided into 5 groups. The most likely gene order is *nic*B-*chl*G-*thi*B-*inl*B. This segment is transferred early in conjugation experiments with Hfr H2 and Hfr B2 as donors. In time-of-entry experiments with Hfr B2 as donor the segment entered about 3 minutes after *pur*C. Consequently this segment maps in the 79- to 82-minutes region of the genetic map. From recombinant analysis of nic^+ recombinants obtained in a four-point cross between Hfr B2 and a *his car*B *pur*C del (*nic chl*G) acceptor the incorporation frequency of the transferred donor fragment was calculated to be about 0.41. The number of crossing-over events per minute length of the chromosome was about the same as in similar crosses between *E. coli* Hfr and F^-. However, between the *nic* and the *pur*C markers it was much higher; it may therefore be inferred that there is a higher probability for a crossing-over event in the regions adjacent to the region that is deleted in the recipient.

In crosses between *S. abony* Hfr H2 del (*nic thi inl chl*) and F^- strains no recombinants were observed which have obtained the deletion from the donor. Nearly all auxotrophic or nic^+ recombinants obtained in a cross between Hfr B2 and a F^- del (*nic*B *thi*B *chl*G *inl*B) strain have inherited all markers of the donor, which are present in the deletion of the recipient.

INTRODUCTION

Deletion analysis has proved very useful for the detection of genes involved in resistance against chlorate in *E. coli* (Adhya, Cleary and Campbell, 1968), *S. typhimurium* (Stouthamer, 1969) and *K. aerogenes* (Stouthamer and Pieters-

ma, 1970). Most of the chlorate-resistant mutants of *S. typhimurium* and of *K. aerogenes*, that do not grow on minimal medium require biotin and have deletions in the *gal-bio* region of the chromosome. However from both organisms a number of auxotrophic chlorate-resistant mutants were obtained, which do not require biotin. Subsequent work has shown that these mutants grow in minimal medium supplemented with thiamin and nicotinic acid (Stouthamer, 1970; Stouthamer and Pietersma, 1970). Furthermore some of these mutants are deficient in the fermentation of inositol. In this paper the order of the markers in this segment will be described and the place of this segment on the genetic map will be given.

MATERIALS AND METHODS

Bacterial strains. The characteristics of the strains used are mentioned in Table 1. Genetic symbols used are those recommended by Taylor and Trotter (1967) and Sanderson (1967). Deleted markers are given in brackets after the symbol del.

Table 1. Characteristics of *S. typhimurium* LT2 and LT7 and *S. abony* strains used

Collection number	Strain	Mating type	Characters	Obtained from
S802	LT7	F⁻	*fer str mut*⁺	Dr. C. Colson
S515	LT2	F⁻	*pur*C7[1]) *his*B *pro*A46 *fla*A56 *str*	Dr. H. Mäkelä
S934	LT2	Hfr B2	*met*A22	Dr. K. Sanderson
S513	*S. abony*	Hfr H2	*met aro str*	Dr. H. Mäkelä
S1056	LT2	F⁻	*thr*302 *pur*C7[1]) *pro*A *ilv rha*461 *fla*A56 *car*B6 *str*	Dr. M. Levinthal
S1057	LT2	F⁻	*thr*302 *pur*C7[1]) *pro*A46 *ilv*405 *rha*461 *fla*A56 *car* B6 *his str*	S1056
S947	LT7	F⁻	*fer str mut*⁺ del (*thi nic chl inl*)	S802
S1058	LT2	F⁻	*thr*302 *pur*C7[1]) *pro*A46 *ilv* 405 *rha* 461 *fla*A56 *fim car*B6 *his str* del (*chl nic*)	S1057
S931	LT2	F⁻	*pur*C7[1]) *his*B355 *pro*A46 *fla*A56 *fim str* del (*thi chl*)	S515
S1053, S1055	*S. abony*	Hfr H2	*met aro str* del (*thi nic chl inl*)	S513

[1]) These strains may contain another mutation in addition to the *pur*C7 marker in the *pur* gene cluster at this place of the genetic map (Stouthamer, unpublished). This is of no consequence for the results however.

Nature of the S. typhimurium LT7 *strains.* Most strains of *S. typhimurium* LT7 carry a mutator gene (*mut*). Since in *S. typhimurium* LT7 *mut* the mutation

frequency towards chlorate-resistance is very high, no chlorate-resistant mutants with deletions could be detected with this strain. Therefore we used *S. typhimurium* LT7 *mut*$^+$. Furthermore these strains have the fertility (*fer*) mutation, which results in the loss of host restriction towards the DNA of *E. coli* (Colson and Colson, 1967; Okada, Watanabe and Miyake, 1968).

Detection of the carB marker. The *car*B marker present in S1056, S1057 and S1058 has been described by Levinthal and Simoni (1969). Mutants with this marker are deficient in the transport of some carbohydrates and consequently these mutants do not grow with glucose, mannitol and a number of other carbohydrates. Growth with galactose, however, is not affected. Therefore the presence of the *car*B marker in recombinants was detected by comparing growth on minimal medium plates with mannitol resp. with galactose as C-source. Levinthal and Simoni (1969) established the gene order *his-car*B-*cys*A-*pur*C. The distance between *car*B and *pur*C was found to be about 5 min.

Medium. The minimal medium was as described by Hadjipetrou et al. (1964).

Isolation of deletion mutants. Chlorate-resistant mutants with deletions were isolated as decribed previously (Stouthamer, 1969; Stouthamer and Pietersma, 1970).

Conjugation experiments. Conjugation experiments were performed as described by Sanderson and Demerec (1965). After interruption of the contact, the mixture was plated directly on selective media without utilization of soft agar. Recombinants to be used for the determination of the linkage frequency of unselected markers or for recombinant analysis were obtained from crosses in which the mating mixture was incubated for 120 minutes.

RESULTS

Properties of deletion mutants. The properties of mutants with deletions in this region of the genetic map are given in Table 2. These mutants could be divided into 5 groups.

Mutants of group 2 have been observed before (Stouthamer, 1969). Whereas mutants with deletions in this region of the genetic map were obtained quite easily with *S. typhimurium* LT7, they were very rare with the LT2 strain. Mutants of group 5 were obtained only with *S. typhimurium* LT2 and with *S. abony*. If we assume that the gene order in these strains is identical, the most likely gene order is *nic-chl*G-*thi-inl*.

Time-of-entry experiments. In conjugation experiments both S513 and S934 transferred this segment early. This indicates that it is part of the 55- to 81-minutes region of the linkage map given by Sanderson (1967). S513 transferred

Table 2. Properties of chlorate-resistant mutants of *S. typhimurium* LT2 and LT7 and *S. abony* with various deletions in the *nic*B-*thi*B region of the genetic map

Strain	Group	Number	Presence of the indicated marker in the deleted region		
			*nic*B	*thi*B	*inl*B
LT7	1	1	+	−	−
S802	2	3	+	−	+
	3	16	−	−	+
	4	14	−	−	−
LT2	1	2	+	−	−
S515	4	1	−	−	−
and S2058	5	6	−	+	*
S. abony	4	2	−	−	−
S503	5	1	−	+	+

The signs − and + denote that the indicated marker is deleted or not, respectively. * The mutants of group 5 from *S. typhimurium* LT2 were derived from S1058. In these mutants the presence of the *inl* marker in the deleted region cannot be determined easily, due to the presence of the *car*B marker in the parent strain.

it within the time needed for contact formation; consequently it must be very close to the origin of Hfr H2.

The results of time-of-entry experiments in which S934 was crossed with a mutant of group 3 (S1058) is shown in Fig. 1. This segment evidently enters the recipient some minutes later than the *pur*C marker. *Pur*C enters 2 à 3 minutes after *car*B. This interval is somewhat shorter than that found by Levinthal and Simoni (1969). These results suggest the order *his-car*B-*pur*C-*nic*.

An experiment with a mutant of group 1 (S931) is shown in Fig. 2. It was impossible in this case, to select for thi^+ recombinants, as the recipient gives a strong background on minimal medium plates without thiamin. Consequently $his^+ thi^+$ and $pur^+ thi^+$ were selected. The experiment of Fig. 1 already showed that $his^+ nic^+$ recombinants appear at the same time as nic^+ recombinants.

The results of the present experiment indicate the order *his-pur*C-*thi*. Consequently it is most likely that the deleted segment maps between the origin of Hfr H2 and *pur*C that is in the 79- to 82-minutes region of the genetic map of *S. typhimurium*.

However the intervals between the times-of-entry of the *car*B, *pur*C and *nic* markers are very short. Although the same results were consistently obtained, we felt the need for confirming the order *nic-pur*C-*car*B in another way, namely by determining the linkage frequency of unselected markers.

On the genetic map of *S. typhimurium* (Sanderson, 1967) *nic*, *thi* and *inl* markers are shown in other regions of the chromosome. Therefore the gene symbols *nic*B, *thi*B and *inl*B are proposed for the markers in the segment studied.

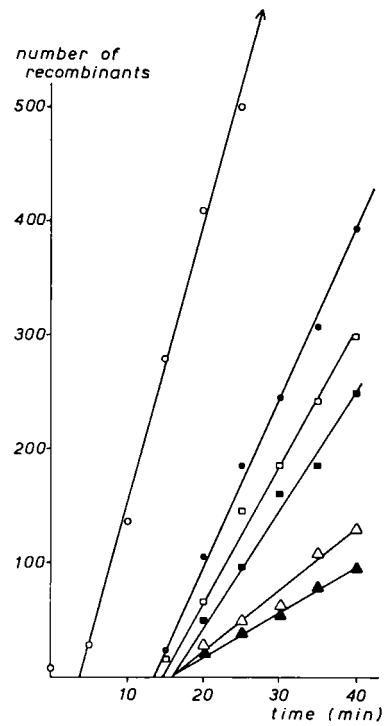

Fig. 1. Kinetics of recombinant formation in an interrupted conjugation cross of S934 × S1058. ○——○ *his*$^+$ recombinants; ●——● *car*B$^+$ recombinants; □——□ *pur*C$^+$ recombinants; ■——■ *nic* recombinants; △——△ *his*$^+$*nic*$^+$ recombinants; ▲——▲ *pur*C$^+$*nic*$^+$ recombinants.

A number of *chl* markers have been observed in *S. typhimurium* (Stouthamer, 1969 and unpublished results). Therefore the gene symbol *chl*G is used for this marker.

Determination of linkage frequencies of unselected markers. *His*$^+$, *pur*$^+$ and *nic*$^+$ recombinants obtained from the crosses S934 × S1058 and S513 × S1058 were analyzed for the inheritance of unselected markers. The results are shown in Table 3. In the cross S934 × S1058 most of the *his*$^+$ recombinants have not

Table 3. Linkage frequencies of unselected donor markers from the crosses S934 × S1058 and S513 × S1058

Cross	Donor marker selected	Number of recombinants	Frequency of donor marker			
			nic	*pur*	*car*B	*his*
S934 × S1058	*nic*	499	1.00	0.57	0.55	0.44
	*pur*C	293	0.67	1.00	0.74	0.48
	his	332	0.19	0.19	0.27	1.00
S513 × S1058	*nic*	100	1.00	0.44	0.38	0.20
	*pur*C	200	0.93	1.00	0.74	0.35
	his	800	0.41	0.29	0.32	1.00

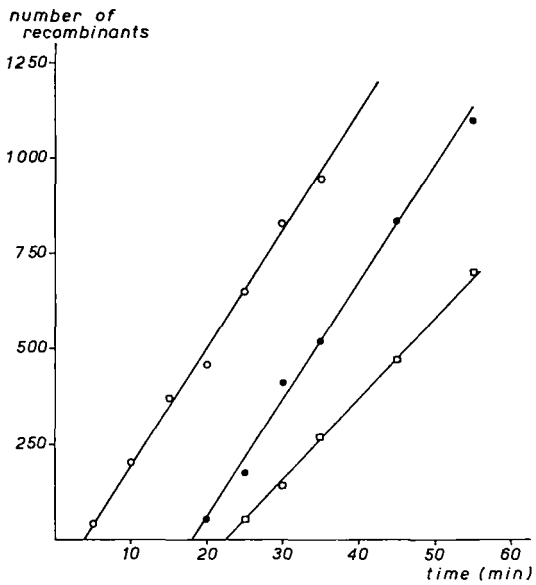

Fig. 2. Kinetics of recombinant formation in an interrupted conjugation cross of S934 × S515. ○——○ *his*$^+$ recombinants; ●——● *pur*C$^+$ recombinants; □——□ *his*$^+$ *thi*$^+$ recombinants.

inherited any of the other markers. This may be explained by the fact that in this cross *his* is a proximal marker. Thus the absence of the unselected donor markers is due to the gradient of transmission. The same applies to the *nic*$^+$ recombinants in the cross S513 × S1058. The data of Table 3 agree with the order *nic-pur*C-*car*B-*his* which we found in the time-of-entry experiments. It is remarkable that in the cross S934 × S1058 the linkage of *nic* and *pur*C is much less than one might expect for markers only 2–3 minutes apart. Most probably this is due to the multisite character of the *nic* marker. Aberrant data are found for the cross S513 × S1058 when *his*$^+$ recombinants are selected. In this cross *his* is the distal marker. We therefore expected that the linkage frequency of the *nic* marker would be lower than those of the *pur*C and *car*B marker. Two kinds of *his*$^+$ recombinants are observed in this cross: large colonies that are *nic*$^+$, and small colonies that are *nic*$^-$. The prevalence of the *nic*$^+$ recombinant is thus most likely due to a selective disadvantage of the deletion mutant. Furthermore many of the colonies were found to contain recombinants of several recombinants classes.

Recombinant analysis. Recombinant analysis is achieved by selecting for a distal donor marker and scoring the selected recombinants for the proximal unselected markers (Jacob and Wollman, 1961). Consequently the distribution of the unselected markers is exclusively due to the recombination process in the zygotes. In the cross S934 × S1058, *nic*$^+$ recombinants were selected and these were scored for unselected markers. The results of this four-point cross are shown in Table 4.

Table 4. Recombinant analysis of *nic*$^+$ recombinants obtained in the cross S934 × S1058

Genotype of recombinants			Number
pur$^+$	*carB*$^+$	*his*$^+$	
+	+	+	121
+	+	−	104
+	−	−	36
−	−	−	112
−	−	+	53
−	+	+	22
+	−	+	23
−	+	−	31
		Total	502

A mathematical model for the calculation of map distances from recombinant analysis has been presented by de Haan and Verhoef (1966) and Verhoef (1968). These authors have established the following relation between linkage and map distance:

$$\beta = \alpha + (1 - \alpha)e^{-kl} \qquad \text{(equation 1)}$$

β = linkage frequency of unselected marker.
α = the probability that a donor fragment is incorporated in the recombinant.
k = proportionally constant per unit length.
l = map distance between selected and unselected markers.

Using this equation de Haan and Verhoef (1966) deduced equations for the frequencies of the eight recombinant classes in a four-point cross. The four parameters (α and three relative map distances) in these equations may be determined from the observed frequencies by means of the method of maximum likelyhood (Bailey, 1961).

The following values have been calculated from the data in Table 4: $\alpha = 0.41$; kl 1 = 1.30, kl 2 = 0.46 and kl 3 = 1.60 (kl 1 = relative map distance between *nic* and *pur*C, kl 2 between *pur*C and *car*B and kl 3 between *car*B and *his*).

When α and the lengths of the three segments have been calculated three degrees of freedom are left to test the fit between the numbers of recombinants found and their expectations in the eight classes. There is a good agreement between observed and calculated frequencies of seven of the recombinant classes. However the observed frequency of the triple crossing-over class (−+−) is twice the calculated frequency. This may be due to the presence of negative interference (Verhoef, 1968). The map distances between *nic* and *pur*C (l 1), between *pur*C and *car*B (l 2) and between *car*B and *his* (l 3) can be determined from the time-of-entry experiments. From the results of Fig. 1 and 2 the values l 1 = 3 min; l 2 = 3 min and l 3 = 11 min are obtained. With these data

the values 0.43; 0.15 and 0.14 can be calculated for k. The last two values are about the same as those found in *E. coli* (de Haan and Verhoef, 1966), however the first one is much higher.

In the same way *his*$^+$ recombinants from the cross S513 × S1058 were scored for the proximal unselected markers. However the observed numbers do not fit satisfactorily with those calculated. This may be due to the selective disadvantage of the deletion mutant.

Recombination between a Hfr *containing the delection and a* F$^-$ *strain.* In crosses between S1053 or S1055 and S515 *pur*C$^+$ and *his*$^+$ recombinants were selected on media supplemented with thiamin and nicotinic acid. Subsequently these recombinants were tested for requirement of these vitamins. None of 200 *his*$^+$ or *pur*C$^+$ recombinants required either. This indicates that in the recombination process in the zygote leading to the production of *pur*C$^+$ or *his*$^+$ recombinants, the deletion of the donor never appears in the recombinants.

*Inheritance of the inl*B *and the chl*G *markers.* In a cross between S947 and S937 *nic*$^+$ recombinants were selected. Five hundred and sixty recombinants were tested for inheritance of the *thi*B, *inl*B and *chl*G markers. All *nic*$^+$ recombinants had also obtained the *thi* marker. Only seven had not obtained the *inl*B marker, four had not obtained the *chl*G marker and one had not obtained either. These results indicate that nearly always the whole donor segment, which is deleted in the recipient, is incorporated.

DISCUSSION

The results show that the *nic*B-*chl*G-*thi*B-*inl*B segment of the chromosome of *S. typhimurium* is most likely situated between the origin of Hfr H2 and the *pur*C marker. On the genetic map of *E. coli*, a *nic*B marker is present on the other side of the *pur*C marker (Taylor and Trotter, 1967). However more recent results suggest that the *nic*B marker has not been placed correctly (Wang, Morse and Morse, 1969). The *nic* marker in the deleted segment of the *S. typhimurium* chromosome may be homologous with the *nic*B of the *E. coli* chromosome. Another *nic* marker was found at the same site as the *nic*A marker of the *E. coli* map (Stouthamer, 1969). In the genetic map of *S. typhimurium* another *nic* marker was mapped between *gal* and *glt* (Sanderson, 1967: Alexander and Calvo, 1969). Consequently three different *nic* markers are known on the *S. typhimurium* map.

A large number of *chl* markers have been found till now. In a previous paper the sequence *nic*A-*aro*G-*gal*-*bio*-*chl*D-*uvr*B-*chl*A was found (Stouthamer, 1969).

Subsequent work using transduction with bacteriophage P22, has shown that at least 4 *chl* genes are present in this segment (unpublished results). Furthermore several *chl* genes have been found in other (still unknown) regions of the chromosome. Till now no *chl* mutants transducible to *chl*$^+$ with P22 have been observed in the region present in the deletion mutants described in this paper (unpublished results). Mutation in all these genes leads to the loss of nitrate reductase A, chlorate reductase C, thiosulfate reductase, tetrathionate reductase and formate dehydrogenase (Stouthamer, 1969 and unpublished results). Recent experiments of Azoulay and Puig (1968) and Azoulay, Puig and Couchoud-Beaumont (1969) show that in *chl* mutants the formation of membranous particles carrying these enzymes is blocked. We may conclude that many proteins are involved in the formation of these particles.

It is known that the total map length of *S. typhimurium* is greater than that of *E. coli* (Sanderson, 1967; Taylor and Trotter, 1967). In the present paper the distances *his-pur*C and *car*B-*his* were found somewhat shorter than in previous studies (Sanderson, 1967; Levinthal and Simoni, 1969). Similarly Alexander and Calvo (1969) found shorter values for the *pur*E-*pyr*D region of the *S. typhimurium* genetic map than the values given by Sanderson (1967). Therefore the total map lengths of the *E. coli* and *S. typhimurium* chromosomes may differ less than has previously been indicated.

It is known that in *E. coli* the incorporation frequency $\alpha = 0.5$ (Jacob and Wollman, 1961; de Haan and Gross, 1962; Verhoef and de Haan, 1966; de Haan and Verhoef, 1966 and Verhoef, 1966). The linkage of unselected proximal donor markers decreases with increasing distance and approaches a minimum value known as genetic equilibrium. The linkage frequency at genetic equilibrium is identical to the incorporation frequency. When in equation (1) kl increases, the value of β approaches asymptotically the value of α. In crosses between *E. coli* Hfr and *E. coli* F$^-$ the linkage frequency at genetic equilibrium is 0.5. Many authors have found however that in *S. typhimurium* Hfr $\times$ *S. typhimurium* F$^-$ crosses the linkage of proximal unselected markers is usually much lower than 0.5 (Sanderson, 1967; Stouthamer, 1969). Sanderson (1967) has found that high linkage is observed in the *str-met*A region of the chromosome. Our results show that the method of de Haan and Verhoef (1966) for the calculation of α and map distances from recombinant analysis in *E. coli* can also be applied to *S. typhimurium*. The value $\alpha = 0.41$ calculated from this four-point cross indicates that in the *his-pur*C segment also high linkage of unselected markers is observed.

The reason for the differences between the linkage frequencies in different segments is not known. De Haan and Verhoef (1966) have shown that in *E. coli* the linkage frequency can be influenced strongly by the mating conditions.

Under unfavourable conditions low linkage frequencies are obtained. Most probably many *S. typhimurium* Hfr × *S. typhimurium* F^- crosses have not been performed under conditions giving optimum linkage frequencies.

We may expect that the presence of a deletion in one of the parents will influence the results of a Hfr × F^- cross since no effective pairing between donor fragment and acceptor chromosome is possible at the place of the deletion. It was indeed impossible to transfer the deletion from the donor to the recipient. Furthermore in crosses in which the deletion was present in the recipient a crossing-over event was frequently observed between the deletion and the adjacent *pur*C marker.

Cases of abnormal recombination in crosses in which a chromosomal aberration or deletion is present in the recipient have been described by Curtiss (1964, 1965). In a number of cases no recombination occurred at all and persisting partial diploidy of a part of the donor genome was observed in these cases.

In a previous paper it was stated that *chl*$^+$ recombinants obtained in a cross between *E. coli* Hfr *chl*$^+$ and *S. typhimurium* LT7 were stable. However after introduction in the recipient of a *gal* mutation which maps very close to the *chl* mutation, the number of *chl*$^+$ recombinants obtained was reduced strongly and furthermore the remaining recombinants were unstable (Stouthamer, 1969). Although it therefore seems clear that in many cases recombinants obtained in crosses in which the recipient has a deletion are partially diploid, no evidence for partial diploidy of recombinants was obtained, however, with the deletion studied in this paper.

Received 12 *March* 1970

REFERENCES

ADHYA, S., CLEARY, P. and CAMPBELL, A. 1968. A deletion analysis of prophage lambda and adjacent genetic regions. — Proc. Nat. Acad. Sci. (Wash.) **61**: 956–962.

ALEXANDER, R. R. and CALVO, J. M. 1969. A *Salmonella typhimurium* locus in the regulation of isoleucine, valine and leucine biosynthesis. —Genetics **61**: 539–556.

AZOULAY, E. and PUIG, J. 1968. Reconstitution of enzymatically active particles from inactive soluble elements in *Escherichia coli* K12. — Biochem. Biophys. Res. Comm. **33**: 1019–1024.

AZOULAY, E., PUIG, J. et COUCHOUD-BEAUMONT, P. 1969. Etude des mutants chlorate-résistants chez *Escherichia coli* K12. I Reconstruction in vitro de l'activité nitrate-reductase particulaire chez *Escherichia coli* K12. —Biochim. Biophys. Acta **171**: 238–252.

BAILY, N. T. J. 1961. Bacterial genetics, chapter 8. *In* Introduction to the mathematical theory of genetic linkage. — Clarendon Press, Oxford.

COLSON, C. and COLSON, A. M. 1967. Host specificity and fertility in *Salmonella typhimurium* LT7. — Biochem. Biophys. Res. Comm. **29**: 692–695.

CURTISS, R. 1964. A stable partial diploid strain of *Escherichia coli*. — Genetics **50**: 679–694.

CURTISS, R. 1965. Chromosomal aberrations associated with mutations to bacteriophage resistance in *Escherichia coli*. — J. Bacteriol. **89**: 28–40.

DE HAAN, P. G. and GROSS, J. D. 1962. Transfer delay and chromosome withdrawal during conjugation in *Escherichia coli*. — Genet. Res. (Cambr.) **3**: 251–272.

DE HAAN, P. G. and VERHOEF, C. 1966. Genetic recombination in *Escherichia coli*. II. Calculation of incorporation frequency and relative map distance by recombinant analysis. — Mutation Res. **3**: 111–117.

HADJIPETROU, L. P., GERRITS, J. P., TEULINGS, F. A. G. and STOUTHAMER, A. H. 1964. Relation between energy production and growth of *Aerobacter aerogenes*. J. Gen. Microbiol. **36**: 139–150.

JACOB, F. and WOLLMAN, E. L. 1961. Sexuality and the genetics of bacteria. — Academic Press, New York.

LEVINTHAL, M. and SIMONI, R. D. 1969. Genetic analysis of carbohydrate transport-deficient mutants of *Salmonella typhimurium*. — J. Bacteriol. **97**: 250–255.

OKADA, M., WATANABE, T. and MIYAKE, T. 1968. On the nature of the recipient ability of *Salmonella typhimurium* for foreign deoxyribonucleic acids. — J. Gen. Microbiol. **50**: 241–252.

SANDERSON, K. E. 1967. Revised linkage map of *Salmonella typhimurium*. — Bacteriol. Rev. **31**: 354–372.

SANDERSON, K. E. and DEMEREC, M. 1965. The linkage map of *Salmonella typhimurium*. — Genetics **51**: 897–913.

STOUTHAMER, A. H. 1969. A genetical and biochemical study of chlorate-resistant mutants of *Salmonella typhimurium*. — Antonie van Leeuwenhoek **35**: 505–521.

STOUTHAMER, A. H. 1970. Genetics and biochemistry of reductase formation in Enterobacteriaceae. — Antonie van Leeuwenhoek **36**: 181.

STOUTHAMER, A. H. and PIETERSMA, K. 1970. Deletion-mapping of resistance against chlorate in *Klebsiella aerogenes*. — Mol. Gen. Genetics **106**: 174–179.

TAYLOR, A. L. and TROTTER, C. D. 1967. Revised linkage map of *Escherichia coli*. — Bacteriol. Rev. **31**: 332–353.

VERHOEF, C. 1968. Recombinant analysis in *Escherichia coli*. — Thesis, Utrecht.

VERHOEF, D. and DE HAAN, P. G. 1966. Genetic recombination in *Escherichia coli*. I. Relation between linkage of unselected markers and map distance. — Mutation Res. **3**: 101–110.

WANG, R. J., MORSE, H. G. and MORSE, M. L. 1969. Carbohydrate accumulation and metabolism in *Escherichia coli*: the close linkage and chromosomal location of *ctr* mutations. — J. Bacteriol. **98**: 605–610.

NOTES ADDED IN PROOF

[1]) The other mutation present in the strains carrying the *pur*C7 marker has been identified as *pur*1590 by Prof. J. S. Gots (personal communication).

[2]) The suggestion that the *nic*B marker has not been placed correctly on the genetic map of *E.coli* (Wang, Morse and Morse, 1970) has recently been confirmed by Tritz, Matney and Gholson (1970). These authors found the gene order *pur*C-*gua*B-*pur*G-*gly*A-*pur*-*nic*B-*tyr*A-*phe*A. These results strongly support the suggestion in this paper that the *nic* marker in the deleted segment of the *S. typhimurium* chromosome is homologous with the *nic*B marker of the *E.coli* chromosome.

TRITZ, G. J., MATNEY, T. S. and GHOLSON, R. K. 1970. Mapping of the *nad*B locus adjacent to a previously undescribed purine locus in *Escherichia coli* K12. — J. Bacteriol. **102**: 377–381.