THE LINKAGE MAP OF *SALMONELLA TYPHIMURIUM*[1]

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Received December 29, 1964

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THREE methods are available for determining long sections of the linkage map of the *Salmonella typhimurium* chromosome: interrupted conjugation (WOLLMAN, JACOB and HAYES 1956), *col*-factor-mediated conjugation (SMITH and STOCKER 1962) and hybridization (DEMEREC 1964; DEMEREC and OHTA 1964). The first method, usually referred to here as kinetic studies, provides the main body of data summarized in this report, whose primary objective is to present a genetic map incorporating all the information we now have about the positions of different gene loci.

S. typhimurium has proved as amenable to analysis by conjugation as *Escherichia coli* K-12, thanks to a method that employs Millipore filters as a solid medium on which effective contact can be made between mating pairs. Kinetic studies made with five Hfr strains having different points of origin are reported here.

MATERIALS AND METHODS

The minimal medium contained: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4$, 0.05 g; $(NH_4)_2SO_4$, 1.0 g; sodium citrate, 0.47 g; glucose, 4.0 g; distilled water, 1000 ml. In minimal medium supplemented with amino acids, purines or pyrimidines, the concentration of supplement was 15 μg/ml; in minimal medium supplemented with vitamins, the concentration was 1.5 μg/ml. Cells were mated in Difco Bacto-Nutrient broth.

Four of the Hfr strains originated from an F^+ strain of *S. typhimurium* LT-2 that carries a sex factor from *E. coli* K-12 (ZINDER 1960b); two were isolated by ZINDER, and two in this laboratory. The fifth strain, a donor strain of *S. abony*, was derived by MÄKELÄ (1963). Properties of the five strains are shown in Table 1.

Almost all the recipient strains were derivatives of *S. typhimurium* LT-2 from the stock collection of M. DEMEREC. Several others were isolated by the authors after treatment with

TABLE 1

Donor strains of Salmonella

Donor symbol	Stock No.	Species	Source	Genotype	Order of injection
HfrA	SR305	*S. typhimurium* LT-2	ZINDER	*his-23 gal-50*	O-*pdx-ilv-thr-try-aroB*
HfrB2	SU422	*S. typhimurium* LT-2	SANDERSON	*metA22 xyl-1*	O-*his-str-thr-try*
HfrB3	SU436	*S. typhimurium* LT-2	SANDERSON	*his-23 gal-50*	O-*argE-his-try-argG*
	SR315	*S. typhimurium* LT-2	ZINDER	*leu-256*	O-*cysB-his-purC*
	SW1452	*S. abony* SW803	MÄKELÄ	*try-291*	O-*metA-ilv-str-purA*

[1] Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

diethyl sulfate designed to obtain polymutant combinations. A few strains were acquired from other laboratories.

These donor and recipient strains mate well when mixed on a solid medium. Effective contacts are rarely formed between male and female cells in a liquid medium; but, following up the report of MATNEY and ACHENBACH (1962) on the use of Millipore filters to increase the fertility of donor strains in *E. coli*, we have found that *S. typhimurium* donors will readily form effective contacts if the mating is started on a Millipore filter.

Overnight broth cultures of donor and recipient strains were diluted 1/40 in broth and grown to mid-log phase on a shaker at 37°C. About 2×10^8 donor cells and 10^9 recipient cells were mixed on a Millipore filter (25 mm diameter, 0.45 μ pore size), the liquid was drawn through the filter under vacuum and the filter was placed on a plate of nutrient soft agar (0.75%) at 37°C for 5 minutes to permit the formation of effective contacts. The Millipore filter was then placed in 16 ml of broth at 37°C in a 250 ml Erlenmeyer flask, the cells were suspended in the broth, and mating was continued with gentle agitation. Gentle washing into broth, gentle agitation and careful pipetting do not separate mated pairs.

For kinetic analysis, time zero was taken as the time at which the cells were drawn onto the Millipore filter. At various time intervals, samples of the mating mixture were diluted in broth, and the mated pairs were separated by shaking for one minute with a vigorous shaking device. (A Fisher Mini-Shaker is used in this laboratory.) Samples were pipetted into 2.0 ml of soft minimal agar (0.75%), which was maintained at 45°C and then poured onto minimal agar plates.

Growth of the donor strains and plate mating were prevented by omitting from the minimal medium the supplements required by the auxotrophic donor strains. In several cases, a streptomycin-resistant mutant of the recipient strain was isolated, and streptomycin (1200 μg/ml) was included in the minimal medium to eliminate the streptomycin-sensitive donor cells.

New donor strains were isolated by the methods described by TAYLOR and ADELBERG (1960).

RESULTS

Isolation of Hfr strains: Eighty-four isolates with Hfr properties were obtained from the ultraviolet-irradiated *S. typhimurium* F^+ strain. Of these, 81 had the point of origin in the same region as HfrA and could not be distinguished from it. The frequency of recurrence of HfrA among F^+ bacteria surviving irradiation was 1 to 5%. Of the three other Hfr strains, two (HfrB2 and HfrB3) served as donors in the experiments reported here (see Table 1).

Kinetic analysis: The point of origin of an Hfr is the first portion of the chromosome to be donated to a recipient strain. On a linkage map, the distance from this point to any gene locus is expressed as the amount of time required for that locus to begin to enter the recipient ("time of entry"). The time of entry is determined by an interrupted-mating experiment, and the distance is expressed in minutes. Data of a representative experiment are shown in Figure 1. The explanation of gene symbols is found in Table 7. In this cross the recipient parent (F^-) was *tyr-3 gly-18 metA*$^+$ and the donor parent was HfrB2 *gly*$^+$ *tyr*$^+$ *metA22*. After mating, the cells were plated on minimal medium supplemented with either glycine or tyrosine. Methionine was omitted to prevent growth of the donor strain. Extrapolation of the curves for *gly*$^+$ and *tyr*$^+$ recombinants to time zero indicated that the *gly*$^+$ locus from the donor parent begins to enter the recipient cells at 31 minutes, and that the *tyr*$^+$ locus begins to enter 4 minutes later. Therefore, the order of genes and the distances between them on the linkage map may be written as O–31–*gly*–4–*tyr*.

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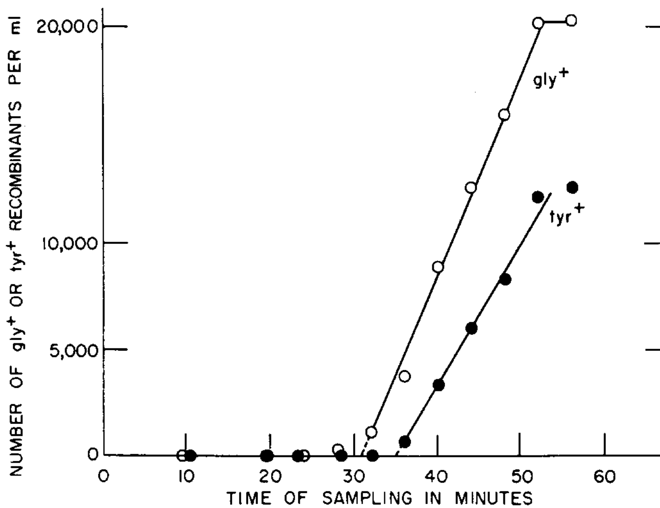

FIGURE 1.—Kinetics of chromosome transfer of donor strain HfrB2.

Data from crosses made with different recipient strains are presented in Tables 2 to 6. The points of origin and the orientation of the five donor strains considered are shown in Figure 2. Each table lists all the kinetic data that have been collected with one of the Hfr strains. Data marked with the same superscript letter are the results of one cross with a multiply auxotrophic recipient. In Table 2, for example, cross "f" showed the time of entry of *argF* to be 15 minutes, that of *thi* 17 minutes. The order of these two loci, and the interval between them on the

TABLE 2

Times of entry, in minutes, of markers transferred by HfrA

pdx	*thrE*	*metE*	*ilv*	*rha*	*asp*	*metB*	*argF*	*metA*	*purD*	*thi*	*purA*	*pyrB*	*serB*	*thr*	*pyrA*
. .	. .	. .	9[b]	. .	13[e]	. .	15[f]	15[b]	. .	17[f]	. .	. .	. .	27[a]	. .
. .	. .	. .	8[c]	. .		. .	. .	15[c]	.	. .	. .	. .	25[i]	26[i]	. .
. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	24[h]	25[h]	. .
8	7	7	. .	13	13	14	. .	. .	15	. .	22	24	. .	. .	28
6	. .	7	. .	. .	. .	14	. .	. .	. .	. .	. .	. .	. .	. .	. .
7	7	7	8	13	13	14	15	15	15	17	22	24	24	26	28

ara	*leu*	*argD*	*pan*	*proAB*	*proC*	*nic*	*purE*	*asc*	*glt*	*pyrC*	*purB*	*aroE*	*cysB*	*try*	*his*
28[e]	29[a]	. .	. .	39[a]	. .	. .	. .	. .	63[l]	67[a]	. .	. .	78[l]	78[a]	. .
29[i]	29[i]	. .	. .	36[g]	. .	. .	43[g]	. .	. .	. .	. .	. .	77[d]	77[d]	86[d]
. .	28[k]	. .	. .	37[h]	. .	. .	46[h]	. .	. .	65[m]	68[m]	. .	. .	77[m]	. .
30	30	32[n]	. .	40[n]	. .	49	. .	45	. .	68	. .	68	. .	76[o]	88[o]
. .	. .	. .	34	37	36	45	. .	. .	. .	67	. .	. .	. .	. .	. .
29	29	32	34	37	36	47	44	45	63	67	68	68	77	77	87

Data carrying the same superscript letter were obtained from a single cross with a multiply auxotrophic recipient.

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TABLE 3

Times of entry, in minutes, of markers transferred by HfrB2

tre	*his*	*metG*	*aroD*	*purG*	*cysA*	*gly*	*purC*	*tyr*	*phe*	*cysC*	*serA*	*argE*	*argG*	*cysG*	*aroC*	*aroB*
..	15[a]	..	..	..	28[c]	30[e]	30[a]	35[e]	38[c]	..	..	..	..	..	..	..
..	15[b]	..	..	..	29[d]	31[f]	30[b]	35[f]	39[d]	39[h]	45[h]	..	..	..	..	..
5[m]	15[m]	..	..	..	..	..	..	33[g]	..	40[g]	45[k]	..	..	..	66[k]	..
8	..	..	..	..	..	..	..	32[i]	..	..	43[i]	52[i]	..	..	..	..
..	15[n]	17[n]	..	..	..	..	32[j]	36[j]	..	..	..	53[j]	..	..	..	..
..	..	14	..	..	..	28[l]	..	34[l]	..	..	..	..	..	..	..	..
..	..	14	23	28	..	29	..	..	..	40	46	..	58	59	68	68
6	15	17*	23	28	28	30	31	34	38	40	45	52	58	59	67	68

Data carrying the same superscript letter were obtained from a single cross with a multiply auxotrophic recipient.
* Cross "n", followed by analysis of the recombinants for unselected markers, clearly showed that *metG* is distal to *his*. Therefore, the two 14-minute times are not considered.

TABLE 4

Times of entry, in minutes, of markers transferred by HfrB3

argE	*metC*	*lysA*	*serA*	*thy*	*argB*	*cysC*	*tyr*	*purG*	*cysA*	*strB*	*aroD*	*try*
13[a]	..	..	18[a]	..	23[c]	24[c]	30[a]	..	..	..	..	..
..	..	..	18[b]	..	22[d]	23[d]	29[b]	..	..	..	..	..
12[g]	..	..	..	19[g]	..	23[e]	30[e]	..	..	..	..	..
..	..	..	..	20[h]	20[h]	..	28[f]	32[f]	..	..	..	..
..	13	16	..	..	..	..	..	..	35	36	39	58
..	13	..	..	..	..	..	..	..	..	..	..	..
13	13	16	18	20	22	23	29	32	35	36	39	58

Data carrying the same superscript letter were obtained from a single cross with a multiply auxotrophic recipient.

TABLE 5

Times of entry, in minutes, of markers transferred by SR315

cysB	*try*	*his*	*purC*
..	10[a]	23[a]	..
9[b]	..	22[b]	..
..	..	..	35
9	10	22	35

Data carrying the same superscript letter were obtained from a single cross with a multiply auxotrophic recipient.

linkage map, may be considered correct, with a possible error of 1 to 2 minutes. However, data with different superscript markings, or with none, are from independent experiments. In such experiments the relative order of loci can be determined only if the average interval between them exceeds 3 to 4 minutes. Thus

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TABLE 6

Times of entry, in minutes, of markers transferred by SW1452

metA	*ile*	*aroC*	*xyl*
..	19[a]	..	30[a]
..	18[b]	..	27[b]
12[c]	.	..	26[c]
..	..	22	..
..	..	25	..
12	18	23	27

Data carrying the same superscript letter were obtained from a single cross with a multiply auxotrophic recipient.

the results of the "f" cross indicate that *thi* is distal to *argF*, but its location with respect to *metA* and *purD* is not definite.

The order of two closely linked loci may be resolved in tests in which the times of entry of both loci are measured in the same experiment and the recombinants are then analyzed for the unselected marker. For example, a strain with mutations in the loci *serB* and *thrA*, previously shown to be carried in the same transducing fragment (GLANVILLE and DEMEREC 1960) was crossed with HfrA $serB^+$ $thrA^+$. Recombinants for $serB^+$ and $thrA^+$ were independently selected in an interrupted-mating experiment. The times of entry were 24 minutes for *serB* and 24.5 minutes for *thrA*. Since these values are too close for reliable determination of the order of the two loci, recombinants selected for $serB^+$ or $thrA^+$ were analyzed for the unselected locus. Among $serB^+$ recombinants selected after 23 minutes of mating, only 61 of 126 (48%) were $thrA^+$, whereas among those selected after 35 minutes, 80 of 82 (98%) were $thrA^+$. All $thrA^+$ recombinants, however, whether selected after 23 minutes (67) or after 35 minutes (99), were $serB^+$. This result shows that *serB* is proximal to *thrA*. In recombinants selected for $serB^+$ after 23 minutes, early interruption of mating prevents the entry of $thrA^+$ into the merozygote, giving rise to a low frequency (48%) of $thrA^+$; but by 35 minutes most merozygotes have received donor alleles for both loci. Therefore, the order of loci suggested by the kinetic data, O-*serB*-*thrA*, is confirmed.

The data of Tables 2 to 6 permit the construction of five linear linkage maps based on the results obtained with the five donor strains. The map compiled by experiments with a single donor strain represents a fraction of the total genome varying from almost one half for HfrA and HfrB2, to about one eighth for SW1452. These independent maps, overlapping one another, make it possible to construct an overall linkage map. In order to fit the five partial maps together, it is necessary to show the final map as a closed circle, like the linkage map of *E. coli* (JACOB and WOLLMAN 1961; TAYLOR and THOMAN 1964).

The linkage map of *S. typhimurium*, 138 minutes in length, begins numbering at *serB* and proceeds clockwise through *pyrB* (Figure 2). A list of all loci on the map is presented in Table 7.

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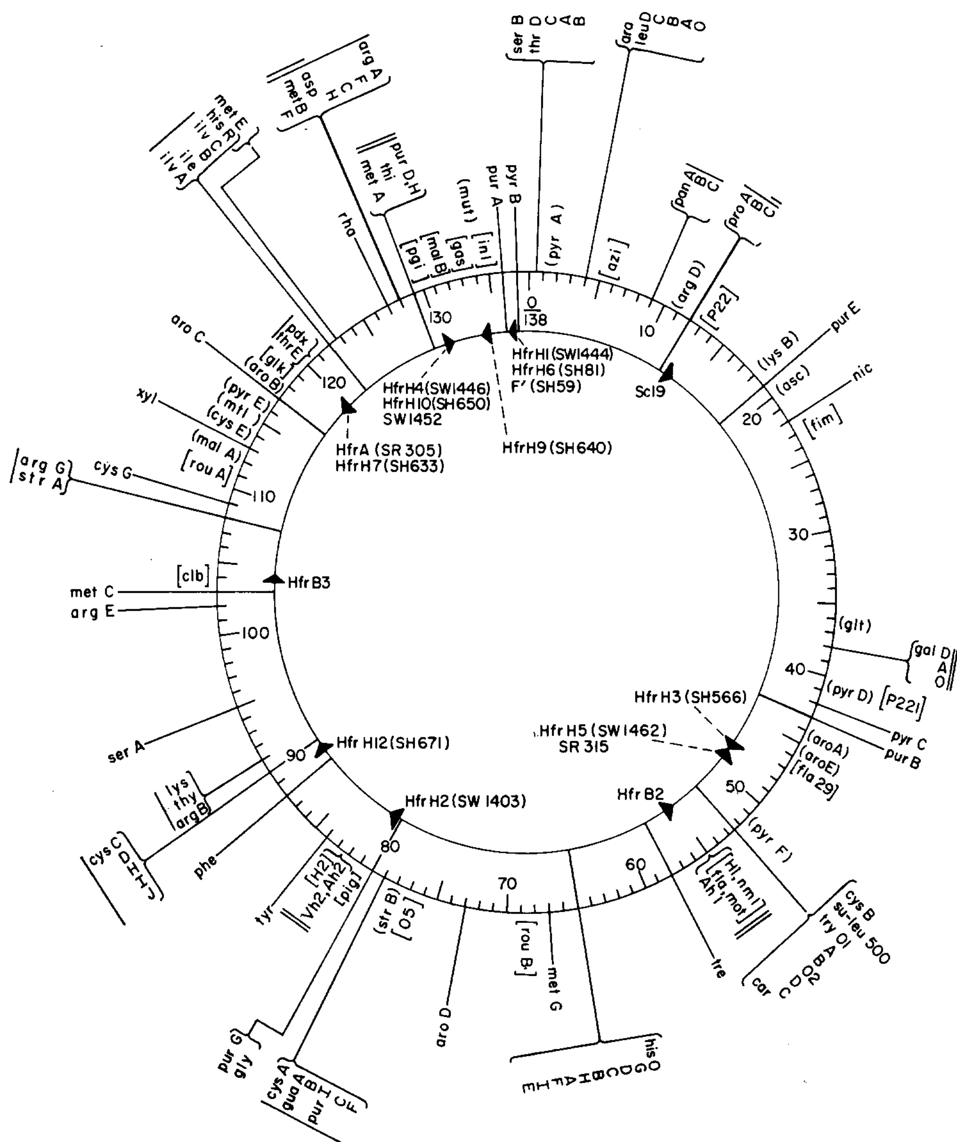

LINKAGE MAP OF SALMONELLA TYPHIMURIUM

} TRANSDUCING FRAGMENT; GENE ORDER & ORIENTATION KNOWN

| } TRANSDUCING FRAGMENT; GENE ORDER KNOWN, ORIENTATION NOT KNOWN

|| } TRANSDUCING FRAGMENT; NEITHER GENE ORDER NOR ORIENTATION KNOWN

( ) POSITION KNOWN APPROXIMATELY

[ ] MAPPED WITH col-FACTOR MEDIATED CONJUGATION

FIGURE 2.—The linkage map of *S. typhimurium*. This map is constructed from the partial maps derived from Tables 2–6, and from other sources given in Table 7. The map is marked in one-minute intervals. Gene loci are shown on the outside circle, the point of entry and direction of injection of donor strains are shown on the inside. HfrA, HfrB2, HfrB3, and SR315 are donor strains of *S. typhimurium*, Sc19 is a *S. typhimurium* × *E. coli* hybrid, and all other donors shown on the map are *S. abony* (MÄKELÄ 1963, unpublished). The location of loci marked by square brackets, determined approximately by *col*-factor-mediated conjugation only, is not known with respect to loci mapped by kinetic studies only. Sixteen other loci shown on the map, which are also listed in the text, have been mapped by *col*-factor-mediated conjugation (STOCKER, SMITH, and SUBBAIAH, 1963) as well as by kinetic studies, and the order determined for these loci by the two methods is identical.

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TABLE 7

List of genetic markers of S. typhimurium

Genetic symbols		Activity	Homologous locus in *E. coli*	References		Map position (min)
				Genetics	Biochemistry	
Ah1		synthesis of flagella of phase 1		36,37	--	55
Ah2		synthesis of flagella of phase 2		36,37	--	82
ara	arabinose	utilization	ara	15	42[*]	4
argB	arginine	N-acetylglutamate synthetase	argB	16	75[*]	91
argC	arginine	N-acetyl-γ-glutamokinase	argC	16	75[*]	128
argH	arginine	N-acetylglutamic-γ-semialdehyde dehydrogenase	argH	16	75[*]	128
argG	arginine	acetyl ornithine-δ-transaminase	argG	16	75[*]	108
argA	arginine	acetyl ornithinase	argA	16	75[*]	128
argD	arginine	ornithine transcarbamylase	argD	16	75[*]	11
argE	arginine	argininosuccinic acid synthetase	argE	16	75[*]	102
argF	arginine	argininosuccinase	argF	16	75[*]	128
asp	aspartate	aspartate or glutamate requirement (phosphoenol-pyruvic carboxylase)[†]		79	73[†]	128
aroA,B,C,D,E	aromatic	aromatic amino acids or vitamins requirement	aroA,B,C,D	55,19	--	45,73,117
asc	ascorbate	requirement		--	--	20
azi	azide	resistance	azi	66	--	6
car	carbohydrate	utilization		52	--	52
clb	cellobiose	utilization		69	--	104
cysA	cysteine	sulfate-thiosulfate permease		51	21,21a	79
cysD	cysteine	adenosine-5'-triphosphate sulfurylase	cysD	13,51	21	90
cysC	cysteine	adenylyl sulfate kinase		13,51	21	90
cysH	cysteine	adenylyl sulfate-3'-phosphate reductase		13,51	21,59,60	90
cysJ	cysteine	sulfite reductase (1st step)		13,51	21,43,59,60	90
cysG,I	cysteine	sulfite reductase (2nd step)		13,51	21,59,60	109,90
cysEa	cysteine	sulfide to cysteine		51	21	114
cysEb	cysteine	sulfate (thiosulfate) to cysteine (activities cysA-cysEa missing)		51	21	114
cysBabc	cysteine	sulfate (thiosulfate) to H_2S (activities cysA-cysG,I missing, cysEa present)	cysB	13,51	21,43,59,60	52
fim	fimbriation			66	--	23
fla29	flagella			66,67	--	47
fla	flagella			66,67	--	55
galO	galactose	operator	galO	24,53	24,53	38
galA	galactose	galactokinase	galA	24,53	24,53	38
galD	galactose	epimerase	galD	24,53	24,53	38
gas		gas production during carbohydrate fermentation		66	--	133
glk	glycerol	glycerol kinase		66	--	119
glt	glutamate	glutamate or proline requirement		79	--	36
gly	glycine	requirement		15	--	80
guaB	guanine	IMP[‡] dehydrogenase (IMP to XMP)	gua	29	--	79
guaA	guanine	XMP aminase (XMP to GMP)	gua	18,29	--	79
H1	antigen	phase 1 flagellar antigen		66,41	45a,48a	55
H2	antigen	phase 2 flagellar antigen		66,41	45a,48a	82
hisO	histidine	operator		1,2	1,44	65
hisG	histidine	phosphoribosyl-ATP-pyrophosphorylase	his	1,33	1,44	65
hisE	histidine	phosphoribosyl-ATP-pyrophosphohydrolase		1,33	1,44	65
hisI	histidine	phosphoribosyl-AMP-hydrolase		1,33	1,44	65
hisA	histidine	isomerase		1,33	1,44	65
hisH	histidine	amido transferase		1,33	1,44	65
hisF	histidine	cyclase		1,33	1,44	65
hisB	histidine	imidazole glycerol phosphate dehydrase and histidinol phosphatase (bifunctional)		1,33	1,44	65
hisC	histidine	imidazole acetol phosphate transaminase		1,33	1,44	65
hisD	histidine	histidinol dehydrogenase		1,33	1,44	65
hisR	histidine	regulator		34,57	44	122
inl	inositol	fermentation		66	--	135

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TABLE 7—*Continued*

List of genetic markers of S. typhimurium

Genetic symbols		Activity	Homologous locus in *E. coli*	References: Genetics	References: Biochemistry	Map position (min)
ile	isoleucine	threonine deaminase	ile	3,26	76	122
ilvA	isoleucine-valine	reductoisomerase	ilvA	3,26	76	122
ilvB	isoleucine-valine	dehydrase	ilvB	3,26	76	122
ilvC	isoleucine-valine	transaminase	ilvC	3,26	76	122
leuO	leucine	operator		52	--	4
leuA	leucine	condensing enzyme	leu	46	40	4
leuC,D	leucine	isomerase		46	32	4
leuB	leucine	dehydrogenase		46	10	4
lys	lysine	requirement	lys	--	--	91
lysB	lysine	lysine + methionine requirement	lys + met	--	4*	18
malA	maltose	utilization	malA	--	--	112
malB	maltose	utilization	malB	66,19	--	132
metA	methionine	homoserine + succinate to o-succinyl homoserine	metA	61,62,19	--	129
metB	methionine	o-succinyl homoserine + cysteine to cystathionine	metB	61,62,19	--	128
metC	methionine	cystathionine to homocysteine	metC	61,62,19	--	103
metE	methionine	homocysteine to methionine (grows on vitamin B_{12})	metE	61,62,19	--	123
metF	methionine	homocysteine to methionine (reduction of folic acid)		61,62,19	--	128
metG	methionine	homocysteine to methionine		62	--	67
mot	motility			66	--	55
mtl	mannitol	utilization	mtl	--	--	115
mut	mutator	increases frequency of mutation		17,49	--	135
nic	nicotinic acid	requirement		--	--	22
nml	N-methyl-lysine	N-methyl-lysine in flagellar protein		66,65a	--	55
O5		synthesis of O antigen 5		66	--	76
P22		PLT-22 prophage site		63	--	13
P221		PLT-221 prophage site		78,80	--	41
panA	pantothenic acid	? to α-ketoisovaleric acid	pan	17	--	9
panB	pantothenic acid	α-ketoisovaleric acid to ketopantoic acid		17	--	9
panC	pantothenic acid	pantoic acid to pantothenic acid		17	--	9
pdx	pyridoxine	requirement		--	--	120
pgi		phosphoglucoisomerase		64	23	130
phe	phenylalanine	requirement	pheA,B	19,55	--	88
pig	pigment	brownish colonies		66	--	81
proA	proline	glutamate to glutamic-γ-semialdehyde	proA,B	50	--	12
proB	proline	glutamate to glutamic-γ-semialdehyde	proA,B	50	--	12
proC	proline	glutamic-γ-semialdehyde to proline	proC	50	--	12
purA	purine§	adenylosuccinic synthetase (IMP to adenylosuccinate)	purA	31,27,28,81	27,28,31	136
purB	purine	adenylosuccinase	purB	27,28,81,31	27,28,31	43
purC,E	purine	conversion of AIR to S-AICAR	purC,E	18,30,81	30	79,19
purD,F,I	purine	early block before AIR	purD	29,81	29	129,79
purG	purine	amidotransferase for FGAR to FGAM		29	9,29	80
purH	purine	AICAR transformylase		29	29,56	129
pyrA	pyrimidine	carbamate kinase (arginine + uracil requirement)	pyrA	79	8*	2
pyrB	pyrimidine	aspartate transcarbamylase	pyrB	79	8*	137
pyrC	pyrimidine	dihydroorotase	pyrC	79	8*	42
pyrD	pyrimidine	dihydroorotic acid dehydrogenase	pyrD	79	8*	41
pyrE	pyrimidine	orotidylic acid pyrophosphorylase	pyrE	79,71*	8*	116
pyrF	pyrimidine	orotidylic acid decarboxylase	pyrF	79	8*	51
rha	rhamnose	utilization	rha	66		127
rouA,B	rough	reduced polysaccharide coat		68	7,54, 52a	110,60
serA	serine or glycine	3-phosphoglycerate dehydrogenase	serA	--	74	95
serB	serine or glycine	phosphoserine phosphatase	serB	26	74	1
strA	streptomycin	high level resistance	str	16	--	108
strB	streptomycin	low level resistance plus auxotrophy		16	--	78
su-leu500		suppressor of leucine 500		52	--	52
thi	thiamine	requirement	thi	--	--	129
thrD	threonine	aspartic acid to β-aspartyl phosphate	thr	26	--	1

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TABLE 7—Continued

Genetic symbols		Activity	Homologous locus in *E. coli*	References		Map position (min)
				Genetics	Biochemistry	
thrC	threonine	β-aspartyl phosphate to homoserine		26	--	1
thrA	threonine	homoserine to homoserine phosphate (homoserine kinase) ?		26	--	1
thrB	threonine	homoserine phosphate to ?		26	--	1
thrE	threonine	? to threonine; grows on isoleucine or threonine		26	--	120
thy	thymine	requirement	thy	22	--	91
tre	trehalose	utilization		69	--	58
tryO1	tryptophan	operator 1		47,52	--	52
tryA	tryptophan	? to anthranilic acid	tryD	5,14	25*	52
tryB	tryptophan	anthranilic acid to indole-3-glycerol phosphate	tryE,C	5,14	25*	52
tryO2	tryptophan	operator 2		47,52	--	52
tryC	tryptophan	tryptophan synthetase	tryA	5,14	25*	52
tryD	tryptophan	tryptophan synthetase	tryB	5,14	25*	52
tyr	tyrosine	requirement	tyr	19,55	--	84
Vh2		control of rate of phase variation		35	--	82
xyl	xylose	utilization	xyl	--	--	113

* These studies were done with *E. coli*.

† The mutant *C⁻dg*^{s}, studied by THEODORE and ENGLESBERG, is probably at the *asp* locus, for like *asp* it is jointly transduced at high frequency with *argA*.

‡ AICAR, aminoimidazolecarboxamide ribotide; AIR, aminoimidazole ribotide; AMP, adenosine-5′-phosphate; ATP, adenosine triphosphate; FGAM, formylglycinamidine ribotide; FGAR, formylglycinamide ribotide; GMP, guanosine-5′-phosphate; IMP, inosine-5′-phosphate; S-AICAR, succinyl-aminoimidazolecarboxamide ribotide; XMP, xanthosine-5′-phosphate.

§ Mutants formerly called *ade* (adenine) and *adth* (adenine-thiamine) have been changed to *pur* (purine). This change was made because the requirement for thiamine has been shown to be a phenotypic manifestation which does not correlate with genotype. *purA*=*adeA*; *purB*=*adeB*; *purC*=*adeC*; *purD*=*adeD*, *adthD*; *purE*=*adeE*; *purF*=*adeF*; *purG*=*adthA*; *purH*=*adthB*; *purI*=*adthC*. The designation for guanine mutants (*gua*) is unchanged (GOTS, unpublished).

Confirmation of the linkage map: The accuracy of the linkage map, constructed originally on the basis of the kinetic data shown in Tables 2 to 6 may be checked by four independent tests, described below.

(1) *Transduction tests*—(a) Loci in the same tranducing fragment should be shown by kinetic tests to be closely linked. Nine transducing fragments that carry genes for unrelated functions were tested. These kinetic tests invariably demonstrated that loci of the same fragment were closely linked, being separated by one minute or less. (b) Loci that are demonstrated by kinetic studies to be widely separated should never be jointly transduced. Many such combinations were tested, and joint transduction was never observed.

Of these nine transducing fragments, three had been previously reported (*try-cysB*, DEMEREC and HARTMAN 1956; *ara-leu* and *serB-thr*, DEMEREC *et al.* 1958), while six were predicted from the completed conjugation map, and established by transduction tests carried out by the laboratory group of M. DEMEREC, according to methods described by GLANVILLE and DEMEREC (1960). The six newly identified transducing fragments are listed below. The numerals joining pairs of loci indicate the percentage of joint transduction. *cysA–guaA*, *B–purI*, *C*, *F* (order not known); *gly*–40–*purG*; *argB*–20–*thy–lys*; *thrE*–42–*pdx*; *metB*, *F*–9–*asp*–70–*argA*, *F*, *C*, *H*; *purD–metA–thi* (order not known).

HARTMAN, ROTH and AMES (1964) and ROTH (personal communication) observed that *hisR* transduced jointly at low frequency while *ile* (1.0%) and with *metE* (2.0%), but that *ile* and *metE* did not transduce jointly. The interpretation is that *ile* and *metE* are on two adjacent fragments which do not have fixed ends, with *hisR* in the overlapping region.

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(2) *Hybridization tests*—An independent method of testing genetic linkage was devised by DEMEREC (1964). Hybrids are produced by conjugation between *S. typhimurium* as recipients and other species of Enterobacteriaceae as donors. Transduction analyses of these hybrids, with *S. typhimurium* auxotrophs as recipients, shows that the "foreign" genetic material of the donor parent usually gives rise to a lower frequency of recombination in *S. typhimurium*. Thus hybrids can serve, in a fashion similar to overlapping deletions, for genetic mapping of the bacterial chromosome. Results of a study involving hybrids from the cross *S. typhimurium* F^- *cysC* × *E. coli* HfrC cys^+ (DEMEREC and OHTA 1964) have shown the following order of loci: *metC—argE—serA—*(*lys-argB*)–(*cysC-D-H-I-J*)–(*phe-tyr*)–*purG–gly*. (Parentheses indicate that the orientation of the group of loci is not known.) These data are consistent with the results of kinetic studies. They permit the ordering of several loci not resolved by conjugation.

(3) *Genetic recombination mediated by colicines*—SMITH and STOCKER (1962) have made genetic analyses of *S. typhimurium* by means of *col*-factor-mediated conjugation. When recombinants for different donor markers are selected, segments of donor chromosome of considerable size and representing every part are transmitted. The order of 16 loci as determined by this method (STOCKER, SMITH and SUBBAIAH 1963; SUBBAIAH and STOCKER unpublished) is as follows: *metA–mal–ara–leu–proA–gal–cysB–tryD–tre–his–purC–cysC–str–xyl–ile–rha*. This order is identical to that independently arrived at by the kinetic studies with Hfr strains. Eighteen additional loci, mapped by STOCKER and associates by the *col*-factor method but not checked by F-factor-mediated conjugation, are shown in square brackets in Figure 2.

(4) *Point of origin of donor strains*—Further support for the correctness of the order of loci derived by kinetic experiments was obtained from a study of the points of origin of five additional donor strains of *S. abony* (SH81, SH640, SH650, SH671, SW1403) provided by MÄKELÄ (1963, and unpublished). With the large number of genetic markers available to us, we were able to determine the points of origin more precisely than was DR. MÄKELÄ (see Figure 2). These strains, and the others shown in Figure 2, will be described in a later publication. For each donor it was established which group of loci entered first and which entered last. The point of origin was between these two groups. The order of loci deduced from kinetic studies was in each case consistent with the order indicated by the position of the point of origin, which was always located between two loci shown by kinetic results to be adjacent.

Six loci were mapped by determining their position in the gradient of recombination frequencies generated by each of the Hfr donors, without the use of kinetic experiments. These loci were *lysB, aroE, malA, cysE, mtl,* and *pyrE*.

Rates of entry of different Hfr strains: A comparison of the results obtained with HfrB2 and HfrB3 indicates that the entry time for the same map interval is very similar in the two Hfr strains, which have opposite polarity but cover the same proximal region. The data for times of entry of the six gene loci that were tested with both strains (see Tables 3 and 4) are summarized in Table 8. The relative order of genes indicated by the two sets of tests is identical, and the time intervals between loci differ very little.

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TABLE 8

Times of entry, in minutes, of markers transferred by both HfrB2 and HfrB3. Time intervals between loci are shown in parentheses

Donor strain	*aroD*		*purG*		*tyr*		*cysC*		*serA*		*argE*
HfrB2	23		28		34		40		45		52
		(5)		(6)		(6)		(5)		(7)	
HfrB3	39		32		29		23		18		13
		(7)		(3)		(6)		(5)		(5)	

DISCUSSION

Enough material is now available for an examination of homology of gene order between *E. coli* (TAYLOR and THOMAN 1964) and *S. typhimurium*. Of the 100 loci shown in the *E. coli* map and 133 on the *S. typhimurium* map, 59 have been sufficiently well analyzed in both organisms to justify the assumption that they are homologous. All of these seem to have identical order on the two maps, and among the other loci only one case of divergence has so far been indicated. This divergence occurs in the chromosomal region concerned with lactose fermentation, which constitutes one of the taxonomic differences between *E. coli* and *S. typhimurium*, *E. coli* being a fermenter and *S. typhimurium* a nonfermenter. Weakly *lac*$^+$ strains have been isolated in Salmonellae (SCHÄFLER and MINTZER 1959; SCHÄFLER, MINTZER and SCHÄFLER 1960), but since the *lac* loci of *E. coli* function normally after transfer into *S. typhimurium* by conjugation, it is probable that these weak *lac*$^+$ strains represent mutations at other loci. SCHWARTZ (1964) presented evidence that in *E. coli* the gene order in the *lac* section of the linkage map is *proA*–(2 min)–*proB*–*lac*–*proC*–(5 min)–*purE*. Except for *lac*, the gene order and distances between genes are similar in Salmonella, and by means of *S. typhimurium* × *E. coli* crosses *lac*$^+$ can be introduced between *proB* and *proC*. It has been suggested (MIYAKE and DEMEREC 1960) that in *S. typhimurium* the *lac* locus has been deleted.

In a few other regions divergence is being investigated. A mutant allele of *E. coli* with the same nutritional phenotype as the *cysA* locus of *S. typhimurium* maps in the same region as the *cysE* locus of *S. typhimurium* (TAYLOR and THOMAN 1964), but since further tests of this allele have shown that it is not biochemically homologous to *cysA* (MONTY unpublished), it does not represent a demonstration of divergence. Differences between the two organisms are seen with respect to map locations of *aro* and *fim* loci, but it is likely that only some of the *aro* and *fim* loci have been mapped in each species and that the mapped loci do not happen to correspond. MÄKELÄ (1964) found that the gene *H1* for the phase-1 flagellar antigen of *S. abony* is allelic to *H*, the gene for the only flagellar antigen of *E. coli*, while the *S. abony* gene *H2* has no counterpart in *E. coli*.

One of the first steps in evolutionary differentiation, as observed in studies of higher organisms, is the appearance of chromosomal rearrangements, particularly inversions. Therefore, the similarity of gene order so far detected between *S. typhimurium* and *E. coli* is highly unexpected. It has been proposed (DEMEREC

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1965) that the responsible factor is the frequent clustering of functionally related genes found in both genera. In order to persist, a gene cluster must offer selective advantage to the organism; thus changes in chromosomal structure that could break a cluster would be disadvantageous to the organism, and would be selected against. The presence of a large number of clusters in a genome would very effectively preserve the existing gene arrangement; and if the evolutionary progenitor of Salmonella and Escherichia possessed such clusters, a high degree of similarity in the gene arrangements of these two bacteria would be expected.

The similarity of the gross genetic maps of Salmonella and Escherichia indicates a close relationship, which is confirmed by the isolation of *S. typhimurium*—*E. coli* hybrids (BARON, CAREY and SPILMAN 1958; DEMEREC *et al.* 1959; ZINDER 1960a). Transduction analyses of such hybrids reveal that the recombination homology between *S. typhimurium* and *E. coli* genetic material within the *cysC* region is only about 1% (DEMEREC and OHTA 1964). It has been suggested that this surprisingly low homology may be due to differences in base sequence of loci that control functionally similar proteins because of (1) degeneracy of the code, and hence the use of different triplets to code particular amino acids, or (2) different amino acid sequences in proteins having the same function.

The map length of *S. typhimurium* is 138 minutes, whereas that of *E. coli* is 89 minutes, only 64% as long. The lengths of 9 map intervals in *E. coli* vary from 39 to 116% of the corresponding intervals in *S. typhimurium* (Table 9). JOHNSON , FALKOW and BARON (1964) also observed longer map intervals in Salmonella but they reported the *E. coli*/Salmonella map lengths for the *ile-metA-proA* intervals to be constant at about 63%, whereas we found 114% and 65% for *ile-metA* and *metA-proA* respectively. They point out that their finding of longer map intervals in experiments with HfrA (SR305) may have been due to differences in mating method, for they did not start matings on Millipore filters and consequently obtained a lower frequency of mating.

Because of the close similarity of the *S. typhimurium* and *E. coli* linkage maps, the greater total map length of *S. typhimurium* is considered to be the result of

TABLE 9

Relative lengths of map intervals in minutes

Interval	*E. coli*	*S. typhimurium*	*E. coli*/*S. typhimurium*
ile-metA	8	7	1.14
metA-proA	15	23	.65
proA-purE	5	6	.83
purE-pyrC	9	23	.39
pyrC-try	4	10	.40
try-his	14	12	1.16
his-serA	18	30	.60
serA-argG	8	13	.61
argG-ile	8	14	.57
Total	89	138	.64

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slower rate of entry rather than greater physical length of the *S. typhimurium* genetic material. Many Hfr strains of *E. coli* and *S. typhimurium* produce "two slope" kinetic data, because they contain two populations with different speeds of entry; the *E. coli* map (TAYLOR and THOMAN 1964) is based on the first slope, the *S. typhimurium* map on the second. Part of the difference in length can be attributed to this fact, but not all, as is shown by instances in which map intervals based on the first slope can be measured in *S. typhimurium*.

The variations in relative *E. coli/S. typhimurium* map distances (Table 9) are thought to be due to variations in rate of entry of the different Hfr strains used to determine the different map intervals in the two bacteria. Differences in length of the genetic material in these map intervals would also explain the data.

The number of transducing fragments carrying known genes is estimated from Figure 2 to be 40 to 60. A range must be given because not all loci shown to be closely linked have been tested for joint transduction. Time intervals between loci in the same transducing fragment (for example, *try cysB, serB thr, ara leu*) are less than one minute. The total number of transducing fragments is estimated as approximately 100 to 150.

The authors gratefully acknowledge the assistance of MR. C. H. HALL, MISS NORIKO OHTA, MISS MARIE ST. PIERRE and MR. LOUIS GLATZER in experimental work, and MISS AGNES C. FISHER for editing the manuscript. They also acknowledge other investigators who supplied details of unpublished mapping results and provided strains.

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SUMMARY

This paper presents a circular linkage map of *S. typhimurium*, 138 minutes in length and containing 133 gene loci, which incorporates all the information we have from experiments done in our laboratory and in others. Key points and many intermediate points were established by interrupted conjugation between recipients and five Hfr strains of *S. typhimurium*. Positions of other markers were determined by transduction, *col*-factor-mediated conjugation, or hybridization. A recently published linkage map of *Escherichia coli* (TAYLOR and THOMAN 1964) contains 100 genes and is 89 minutes long. Studies of 59 loci that are mapped in both organisms have been sufficiently thorough to permit the conclusion that these loci have the same function in *S. typhimurium* and *E. coli*. On the maps all 59 are located in similar positions. Only one case of gene divergence is suggested; the *lac* loci of *E. coli* may be missing from *S. typhimurium*. These findings indicate a high degree of homology in gross genetic structure. The number of transducing fragments carrying identified genes is estimated as 40 to 60.

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