GENETIC ANALYSIS OF THYMINELESS(*thy*) MUTANTS IN *SALMONELLA TYPHIMURIUM*[1]

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AMINOPTERIN may be used to select readily thymineless (*thy*) mutants in *Escherichia coli* (OKADA, HOMMA, and SONOHARA 1962); however, this selection is more difficult in *Salmonella typhimurium* strain LT2. Since this may be due to the rarity of *thy* mutation in this organism, the mutagen N-methyl-N′-nitro-N-nitrosoguanidine (NG) was combined with aminopterin to successfully mutate and select *thy* mutants (NISHIOKA and EISENSTARK 1964).

Approximately 300 *thy* mutants of *S. typhimurium* were collected (Table 1) and many of these were analyzed, seeking answers to these questions: (1) What is the precise position of *thy* on the chromosome map of *S. typhimurium* (see SANDERSON and DEMEREC 1965 for detailed map)? (2) What loci are included in the same phage P22 transducing fragment with *thy*? (3) What is the clockwise map orientation of the *thy* locus and its neighbors? (4) Do all *thy* fall into a single locus? (5) Are all *thy* physiologically identical? (6) By reversion tests with a series of mutagens, which *thy* appear to be single-site and which multisite mutants? In the course of analyses, a number of observations led to additional probing of the degree of heterogeneity of transducing particles, sensitivity of cells to deoxyribosides, and the mapping of neighbors of *thy*.

While this investigation was in progress, a report appeared (ALIKHANIAN *et al.* 1966) of similar experiments with *E. coli*.

MATERIALS AND METHODS

Conjugation methods for *S. typhimurium* were presented by SANDERSON and DEMEREC (1965). Most other materials and methods were described by DEMEREC *et al.* (1956), and special materials and techniques are presented in RESULTS.

Induction and selection of thy *mutants:* A series of liquid cultures was inoculated with a small number (ca. 10^3–10^4) of *S. typhimurium*-LT2 cells. The medium contained thymine (200 μg/ml), aminopterin (200 μg/ml), NG (3 μg/ml), K_2HPO_4 (10 μg/ml), KH_2PO_4 (4.5 μg/ml), $(NH_4)_2SO_4$ (1 μg/ml), sodium citrate (0.5 μg/ml), $MgSO_4$ (0.05 μg/ml) and glucose (4 mg/ml). The salts were prepared in one batch, and each of the other components was prepared separately; all ingredients were mixed prior to use. NG and aminopterin were not sterilized.

Two days of incubation were required before turbidity appeared in the broth cultures. A loopful of grown culture was taken from each of the tubes and passed through single colony isolation on minimal + thymine (0.5 to 5 μg/ml) agar plates. Aminopterin and NG were omitted from the agar. Plates were incubated overnight. About 1 to 10% of the colonies were *thy*⁻ and

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could be detected by their transparent appearance and smaller size. Since each plate contained cells from only one of the series of tubes that were inoculated initially, mutants selected from different plates represented independent mutational events; thus each *thy*$^-$ in the collection should be different.

Variation of the amount of thymine in the agar gave somewhat different results. At the lowest concentration (0.5 μg/ml) *thy*$^-$ colonies were very small, and only about half of them contained viable cells. Apparently, many of these cells had undergone thymineless death. At the other extreme of 5.0 μg/ml, *thy*$^-$ colonies were much larger but they could still be distinguished from wild type by their transparent appearance. All such colonies were viable, but about 10–20% contained wild-type cells and had to be purified further. A compromise of 3.0 μg/ml was found to work very well for *S. typhimurium*.

When the mutants were collected initially, it was not recognized that *thy* mutants fall into two classes: those that can grow on low amounts of thymine (ca. 2 μg/ml) and those that require a larger amount (ca. 20 μg/ml). However, representatives of both classes appeared in the collection (Table 1).

In one set of experiments, 5-bromodeoxyuridine (200 μg/ml) was used as a mutagen and compared with NG. NG was found to be far more efficient in the induction of *thy* mutants. Also, some of the *thy* mutants were obtained by ultraviolet (UV) treatment.

Transduction: An overnight culture of recipient bacteria (2×10^9 cells/ml.) was infected with phage P22 grown previously on donor bacteria. The multiplicity of infection was five plaque forming units (pfu) per bacterial cell. The mixture was incubated at 37°C for 15 minutes, after which 0.1 ml was spread on each agar plate. Colonies were scored after 48 hours of incubation at 37°C.

It is difficult to predict, with any degree of precision, the number of transductants that one may obtain in any given experiment. Variation in the frequency of lysogenic and lytic responses may account for much of the uncertainty. To check whether such factors would influence joint transduction ratios (Tables 2, 4, 6 and 7), two confirmatory experiments were performed. In the first, lysogenic cells were used as recipients. While the number of transductants was only about 10% of that obtained with non-lysogenic recipient cells, no difference in ratio of two linked markers was detected. In the second confirmatory experiment, antiserum against phage P22 was added to agar plates used for the transduction procedures (k value of antiserum in agar = 0.1/plate) to prevent secondary infection by phage released from bursts of cells on plates. Again, no difference in ratio of two linked markers was detected in joint transduction experiments. Thus, while the number of transductants varies, the ratio of two linked markers among transductants is constant as long as the same donor phage stock is used.

Reversion tests: Auxotrophs (ca. 2×10^8 cells per plate) were spread on plates containing minimal agar with 0.01% nutrient broth powder. The medium permits only a few divisions of the *thy* auxotrophs but allows revertants to grow into colonies. The mutagens were added to the surface of inoculated plates as small droplets; revertants arose as a zone of colonies around the point of mutagen addition (EISENSTARK and ROSNER 1964). Because of the large number of auxotrophs that were tested, only diethyl sulfate and nitrosoguanidine (EISENSTARK, EISENSTARK and VAN SICKLE 1965) were used as mutagens. This was intended only as a screening procedure, and it is recognized that further testing, including the use of additional mutagens, might place some mutants into different categories (Table 1). Also, it is recognized that the term "no revertants" means that no colonies were seen on minimal plates containing ca. 10^{10} cells after incubation; upon increasing the number of cells examined, it is possible that revertants might arise.

RESULTS AND DISCUSSION

On initiation of this problem, the position of *thy* in *S. typhimurium* was unknown. Recently, *thy* in *E. coli* has been mapped and found to be in the same region as we have found for *S. typhimurium* (ISHIBASHI, SUGINO and HIROTA 1964; ALIKHANIAN, *et al.* 1966).

TABLE 1

Thymineless (thy) *mutants of* S. typhimurium; *grouped according to reversibility and the presence of a second mutation to low thymine requirement* (tlr)

No reversions				Reversions to small colonies only		Spontaneously reversible; mutagen stable		Spontaneously stable; mutagen reversible		Reversible spontaneously and by mutagen			
tlr+		*tlr*−		*tlr*+	*tlr*−	*tlr*+	*tlr*−	*tlr*+	*tlr*−	*tlr*+		*tlr*−	
thy 52,		*thy* 176,		*thy*	*thy*	*thy*	*thy*	*thy*	*thy*	*thy* 102,		*thy*	
53,	54,	195,	247,	82,	90,	66,	144,	94,	119,	103,	109,	104,	
55,	56,	257,	260,	83,	271,	67,	164,	96,	123,	110,	111,	105,	
57,	58,	262,	265,	86,	288,	70,	173,	100,	124,	112,	113,	165,	
60,	61,	272,	275,	87,	297,	73,	564,*	101,	128,	114,	115,	170,	
62,	63,	329,	330,	88,	299,	77,	572,*	127,	136,	117,	125,	181,	
64,	68,	339,	341,	92,	300,	80,	639,*	129,	137,	126,	182,	192,	
69,	71,	347,*	392,*	98,	306,	81,	653,*	130,	143,	197,	202,	276,	
72,	74,	398,	561,*	213,	307,	85,	656,*	131,	145,	203,	224,	281,	
75,	79,	562,*	570,*	216,	309,	142,	692 *	132,	149,	226,	227,	302,	
84,	91,	589,*	591,*	219,	563,*	199,	...	134,	150,	231,	233,	304,	
93,	106,	593,*	600,*	223,	576,*	200,	...	138,	152,	235,	236,	305,	
148,	168,	637,*	643,*	237,	577,*	206,	...	139,	154,	239,	240,	308,	
174,	189,	646,*	651,*	238,	603,*	211,	...	140,	157,	242,	243,	320,	
193,	282,	657,*	666,*	248,	639,*	241,	...	141,	158,	250,	251,	322,	
283,	284,	675,*	678,	249,	648,*	245,	...	147,	179,	254,	255,	566,*	
285,	286,	679,	681,*	261,	663,*	253,	...	151,	196,	256,	259,	586,*	
287,	289,	683,	691,*	264,	665,*	268	...	155,	198,	269,	270,	650,*	
290,	291,	694,*	695,*	266,	668,*	...	...	156,	568,*	319,	332,	669,*	
292,	293,	700,	706,	267,	674,*	...	...	159,	569,*	340,	354,	701,	
294,	295,	707,	718 *	273,	685,*	...	...	177,	574,*	355,	360,	703,	
298,	331,	...	...	274,	687,	...	...	184,	575,*	364,	366,	709,	
676,	680,	...	...	278,	689,	...	...	187,	644,*	721	...	711 *	
682,	697,	...	...	279,	693,*	...	...	191,	652,*	...	...	...	
699,	713	...	...	280,	702,	...	...	194,	658,*	...	...	...	
...	...	...	...	296,	704,	...	...	207,	662,*	...	...	...	
...	...	...	...	338,	708,	...	...	209,	715 *	...	...	...	
...	...	...	...	684,	714	...	...	218,	...	...	...	...	
...	...	...	...	686,	...	...	...	220,	...	...	...	...	
...	...	...	...	696,	...	...	...	221,	...	...	...	...	
...	...	...	...	705,	...	...	...	222,	...	...	...	...	
...	...	...	...	710	...	...	...	232	...	...	...	...	

* Denotes strains that are highly sensitive to deoxyribonucleosides. All other *thr* strains are also sensitive to deoxyribosides, but to a lesser degree.

The mapping of *thy* in *S. typhimurium* was done in three steps, each providing a different degree of localization. This was done for a number of *thy* mutants since we wanted to insure that a possible second locus for *thy* would not be overlooked.

Rough mapping of 80 thy *mutants by conjugation spot tests:* Initially, the general location of *thy* was identified by spotting different Hfr strains (Figure 1) on minimal plates seeded with 80 different *thy* recipients. Since the point of chromosome entry differed in various Hfrs, it was assumed that spots that con-

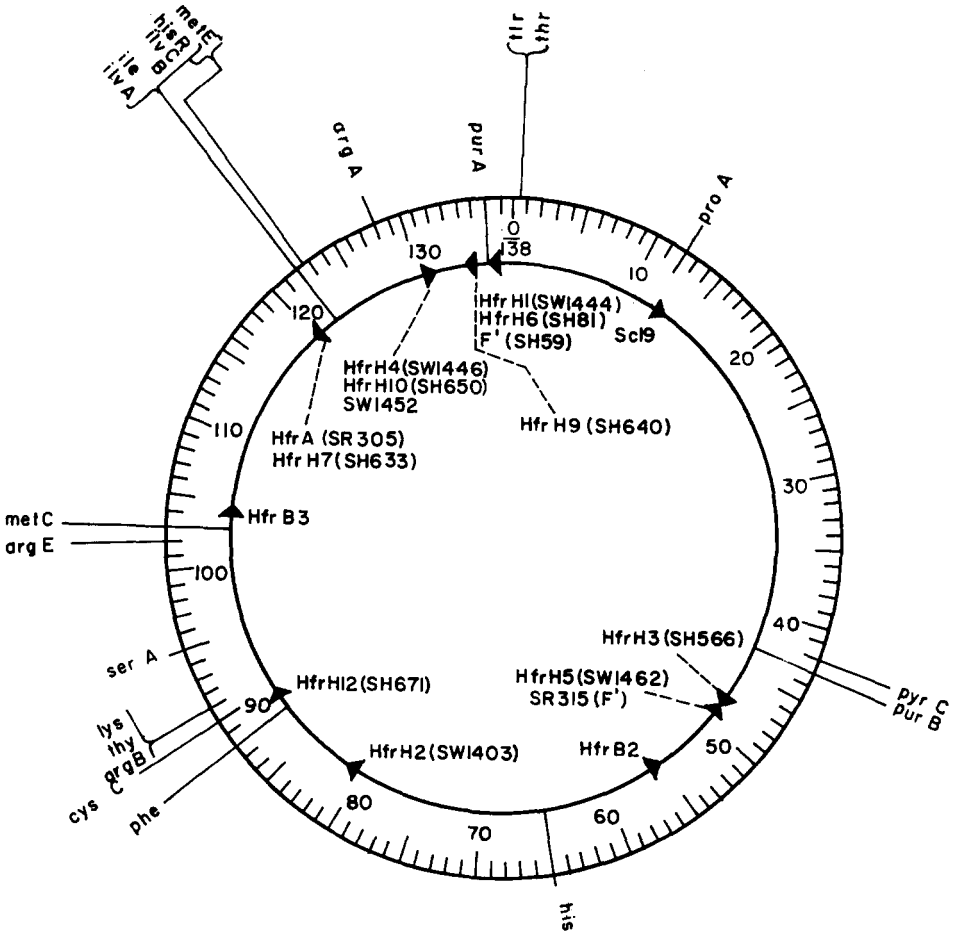

FIGURE 1.—Map of *S. typhimurium* chromosome (after SANDERSON and DEMEREC). Donor strains are indicated inside of circle, with points of origin and direction.

tained high numbers of prototrophs (recombinants) identified the Hfr in which *thy*$^+$ was an early marker. Also, Hfr × *thy* F$^-$ crosses giving no, or few, recombinants, identified the Hfr in which *thy* was a late marker. When crosses were made, all 80 *thy* that were examined were in the general region between *cysC* and *metC* (between entry points of Hfr B3 and SH671, Figure 1). HfrB2 and HfrB3 gave more recombinants than those Hfrs which had points of origin distant from this region; SH671 gave no recombinants, and thus it was concluded that *thy* must be a very late marker.

Since all 80 *thy* mapped in the same region, it was concluded that aminopterin selection yields only *thy* mutants that map in this region.

Localization of thy *position by time of entry (conjugation) experiment:* With the knowledge that *thy* was located between *metC* and *cysC*, *thy* was induced in the existing mutants *metC52*, *argE116*, *serA13*, *lys6*, and *argB69* and these double

mutants were used in time of entry experiments. The results showed that the entry time of the *thy* allele was about the same as for *argB* and *lys*, but different from all of the others; therefore, *thy* is closely linked to these two loci.

Pinpointing the thy *locus by joint transduction:* Up to this point, the orientation of *thy* with respect to *argB* and *lys* was not known. The orientation was determined by a series of co-transduction tests in which several *thy*, *argB69*, *lys6*, and double mutants were used in crosses. By such analysis, it was found that *thy* co-transduces with both *lys6* and *argB69*, and that it lies between the two (Table 2), thus answering questions 1 and 2 above, as to the position of *thy* on the genetic map.

Linkage of lys-thy-argB *in episome of* E. coli: Further evidence of linkage of these three loci came by transferring episome F15 from *E. coli* W4580 (kindly supplied by Y. HIROTA) to a series of auxotrophic mutants of *S. typhimurium*. After crossing with *E. coli* W4580, it was found that prototrophs arose only in *thy*, *lys* or *argB* mutants (or double or triple mutants of these), but in no other auxotrophs including those with adjacent markers *serA* and *cysC* (Table 3). Until markers are found between *serA-lys* and between *argB-cysC*, the ends of episome F15 may be set temporarily at *lys-argB*. The linkage of *thy* and *arg* in F15 in *E. coli* was reported by ISHIBASHI, SUGINO and HIROTA (1964).

Clockwise orientation of loci: Conjugation data (Table 4) indicate that the clockwise order may be *argB-thy-lys*. This is based on the assumption that, if zygotes are analyzed shortly after recipients receive a donor marker, a marker immediately preceding this will be incorporated along with it in a high percen-

TABLE 2

Joint transduction of lys, thy *and* argB *loci*

Donor	Recipient	Selected marker	Unselected marker	% Transduction of unselected to selected marker
Wild type				
	lys6 thy330	*lys*+	*thy*+	18%
		thy+	*lys*+	37%
	thy341 argB69	*thy*+	*argB*+	38%
		argB+	*thy*+	10%
	lys6 argB69	*lys*+	*argB*+	0.1%
		argB+	*lys*+	0.1%
lys6⁻thy+*argB*+				
	thy283	*thy*+	*lys⁻*	39%
	thy341 argB69	*argB*+	*lys⁻*	1.6%
lys+*thy*+*argB69⁻*				
	thy283	*thy*+	*argB⁻*	30%
	lys6 thy330	*lys*+	*argB⁻*	5%

NOTE: Co-transduction tests of *serA13 lys6*, *serA13 thy342*, *argB69 cysC1021*, and *thy664-cysC1021* were all negative (*serA* and *cysC* straddle *lys thy argB*). The percentages of joint transduction of *lys* and *argB* (1.6–5%) are higher when there is a *thy⁻* in the recipient than when a recipient is *thy*+.

TABLE 3

Wild-type colonies found on crossing E. coli *W4580 (Episome F15) and* S. typhimurium strains 10^8 *recipients* (0.1 ml) *mixed with* 10^7 *donors and spread on minimal agar plate*

Recipient	Number of wildtype colonies
metC 52	0
argE 116	0
serA 13	0
lys 6	1082
thy 283	946
argB 69	1161
cys C 1021	0
lys 6 thy 330	941
thy 341 *argB* 69	1060
lys 101 *thy* 592 *argB* 69	581

tage. On the other hand, if selection is made for the first of the two markers, the percentage of zygotes that receive the second marker will be lower. Data in Table 4 indicate a clockwise order of *argB-thy-lys.*

Do all thy *fall into a single locus?* None of 128 *thy* examined thus far have been located outside of *lys6-argB69*, although a second location has been sought near *argE* where its map position had been indicated earlier in *E. coli* (see map by TAYLOR and THOMAN 1964). More recently, ALIKHANIAN, *et al.* (1966) and OKADA (1966) have demonstrated *thy* location close to *cys* and *ser* in *E. coli.* The possibility exists that there is more than one *thy* locus, but that these are clustered. Complementation tests by abortive transduction have been negative, but complications of thymine starvation cannot be ignored. It should not be forgotten that aminopterin may select only one functional type among cells that have mutated at more than one *thy* locus.

TABLE 4

Determination of clockwise order of arg B, thy *and* lys *loci on genetic map of* S. typhimurium *by interrupted mating experiment with* argB69 thy 341 *recipient and Hfr B3 donor**

Time of interruption of mating	Selected marker	Number of recombinants observed	% transfer of both markers
20 min.	*argB*+	34 *argB*+ *thy*+	100%
		0 *argB*+ *thy*−	
60 min.	*argB*+	331 *argB*+ *thy*+	100%
		0 *argB*+ *thy*−	
20 min.	*thy*+	27 *argB*+ *thy*+	77%
		8 *argB*− *thy*+	
60 min.	*thy*+	209 *argB*+ *thy*+	84%
		41 *argB*− *thy*+	

* Data from cross of *lys*− *thy*− recipient × Hfr B3 are in agreement with and confirm order of *argB thy lys.*

WILSON, FARMER and ROTHMAN (1966) stated that in *B. subtilis* there are two, non-linked *thy* loci, *thy A* and *thy B*; *thyA*$^-$*thyB*$^+$ and *thyA*$^+$*thyB*$^-$ both have *thy*$^+$ phenotype. They concluded that *thyA* involves the enzyme thymidylate synthetase, but that *thyB* involves an enzyme that allows thymine synthesis via an alternate pathway.

If *S. typhimurium* has two such *thy* loci, they must be closely linked, unlike the situation in *B. subtilis*. The following points give evidence that in *S. typhimurium* they could not be distant from each other: (1) Conjugation with Hfr strains of different entry points and direction showed no indication of a *thy* locus in a neighborhood other than the one designated on Figure 1. (2) If there are two *thy* loci in *S. typhimurium*, they are closely linked and on the same transducing fragment. Otherwise, it should not be possible to transduce *thy*$^-$ into recipients that are *thy*$^+$. That such transductions may be accomplished readily may be seen from Table 2.

This still leaves the possibility that two closely linked loci, similar to *thyA* and *thyB* in *B. subtilis*, are present in *S. typhimurium*. If so, one would predict different relationships in joint transduction between *thy* and its *lys* and *argB* neighbors, depending on the choice of donor and recipient phenotypes. That is, if only one of the two loci (*thyA*$^-$ or *thyB*$^-$) need be changed to *thy*$^+$ to give *thy*$^+$ phenotype, co-transduction of one *thy* with a neighbor would be more frequent than if both *thy* had to be co-transduced with that neighbor. Table 5 shows that such results were actually obtained; transduction of the hypothetical *thyA*$^-$*thyB*$^-$*argB*$^-$ recipient to give the *thy*$^+$ *argB*$^+$ recombinant phenotype was higher than transduction of hypothetical *thyA*$^+$*thyB*$^+$*argB*$^-$ to give the *thy*$^-$*argB*$^+$ phenotype. A cautious note should be added: while this is suggestive of a second *thy* locus, more direct proof is needed, especially since transduction of the *thy* locus occurs at a low frequency (see below). The differences in the crosses described in Table 5 could have an explanation other than the existence of two *thy* loci.

Another indication that there may be two *thy* loci is the fact that not all *thy*$^+$ revertants are identical. As WILSON, FARMER and ROTHMAN (1966) found in *B. subtilis*, some *thy*$^+$ revertants of *S. typhimurium* remain aminopterin resistant (AMP^r). It is possible that AMP^r revertants do not have the enzyme thymidylate synthetase and use an alternate pathway, but enzyme assays have not been performed on these *S. typhimurium* revertants.

Crossfeeding tests have shown no sign of complementation. Again, thymineless death may exclude the feasibility of crossfeeding tests. Wild-type *S. typhimurium* does not feed *thy* mutants.

Physiological types: Thymineless mutants may be separated into two distinct groups: some require only 1–3 μg/ml, others require 20–50 μg/ml. Both types were found among the initial *S. typhimurium* isolates; of 297 *thy* mutants tested, 172 were of the first type and 125 were of the second. In *E. coli*, it is now known (HARRISON 1965; ALIKHANIAN *et al.* 1966; OKADA 1966) that mutants with low thymine requirement are actually double mutants; a second mutation allows cells to grow at only *ca.* 2 μg/ml. The same is true for *thy* mutants of *S. typhimurium*, and details of the physiological differences between the two types are

TABLE 5

Comparison of expected and actual numbers of joint transductants, assuming two linked thy *loci*, thyA *and* thyB

Recipient	Donor	Phenotype	Joint transductions Expected %	Actual %	Genotype of joint transductants
(thyA⁻thyB⁻)argB⁻ (thy341 argB69)	*(thyA⁺thyB⁺)argB⁺*	*thy⁺argB⁺*	X	16%	Could be either *thyA⁻thyB⁺argB⁺* or *thyA⁺thyB⁺argB⁺*
(thyA⁺thyB⁺)argB⁻ (argB69)	*(thyA⁻thyB⁻)argB⁺ (thy 283)*	*thy⁻argB⁺*	less than X	6%	Must be *thyA⁻thyB⁻argB⁺*
lys⁻(thyA⁻thyB⁻) (lys 6 thy330)	*lys⁻(thyA⁺thyB⁺) (lys 6)*	*thy⁺lys⁺*	Y	9%	Could be either *lys⁺thyA⁺thyB⁺* or *lys⁺thyA⁺thyB⁻*
lys⁻(thyA⁺thyB⁺) (lys 6)	*lys⁺(thyA⁻thyB⁻) (thy 283)*	*thy⁻lys⁻*	less than Y	3%	Must be *lys⁺thyA⁻thyB⁻*

reported elsewhere (BEACHAM, BARTH, EISENSTARK and PRITCHARD, in preparation).

Genetically, the mutation for thymine low requirement (*tlr*) is found to be linked to threonine (Figure 1) as determined by crossing *thy* 2454 *thrA*8 F^- with SH59 donor. ALIKHANIAN *et al.* (1966) and OKADA (1966) found *tlr* to be linked to *thr* in *E. coli* also. Our attempts to co-transduce *tlr* with *thr* or *leu* in *S. typhimurium* were not successful; thus its precise location has not been determined.

Thymidine sensitivity: ALIKHANIAN *et al.* (1966) reported that some *thy* mutants of *E. coli* are sensitive to high concentrations of thymidine, and they termed these thd^s mutants, with close linkage to *tlr* and *thr*. Many of our *S. typhimurium tlr* mutants were thymidine sensitive, and also mapped in the same region. However, we found a striking difference. In crosses with Hfr donors, and in transduction tests, 100% of our recombinants simultaneously were changed from tlr^-thd^s to tlr^+thd^r. This would indicate extremely close linkage of the *tlr* and thd^s loci. More striking, however, in tests of several hundred revertants of thd^s, 100% of them also had been changed from tlr^- to tlr^+. This indicated that the same gene was responsible for both phenotypic characters. Detailed studies of deoxyribonucleoside-sensitive mutants of *S. typhimurium* mutants will be reported elsewhere (BEACHAM, BARTH, EISENSTARK and PRITCHARD, in preparation). An interesting aspect is that cells need not be thy^- to exhibit thymidine sensitivity. When thy^+ revertants were examined, they were equally as sensitive to thymidine as their thy^- predecessors.

Upon further testing, it was found that all *tlr* mutants exhibited some sensitivity to thymidine and other deoxyribosides, but there were two quantitative groups. One group was sensitive to as little as 5 μg/ml of deoxyriboside (designated with asterisk in Table 1), whereas the remaining *tlr* mutants required larger amounts of deoxyribosides to exhibit sensitivity. As in *E. coli* (DALE and GREENBERG 1967), the highly sensitive mutants are missing the deoxyribose aldolase, and the less sensitive *tlr* mutants lack the deoxyribose mutase (BEACHAM, BARTH, EISENSTARK and PRITCHARD, in preparation). These map as linked loci in *S. typhimurium*.

Possible heterogeneity of thy *transducing fragment:* In all experiments, it was puzzling that the transduction frequency for *thy* was always lower by a factor of 4–10 than that for the neighboring markers *lys* and *argB*. Since *thy* is obviously between *lys* and *argB*, transduction of *thy* should be *higher* rather than *lower*, since crossovers should be more frequent in the center of a transducing fragment than at the ends, assuming homogeneity among *thy* transducing fragments (OZEKI 1959). Complications of thymine metabolism, including thymineless death, could not explain low thymine transductions, since: (1) when double auxotrophs (i.e., *thy argB*) were used as recipients in the same experiment, transduction of *argB* remained high as compared with *thy*; (2) transduction of *thy* remained low regardless of whether phage and bacteria were added to minimal plates directly or whether phage and bacteria were mixed and pre-incubated in thymine-enriched medium for periods as long as two hours; (3) in conjugation tests, the numbers of thy^+ recombinants were as high as for lys^+ and $argB^+$. One

possible explanation is that there is heterogeneity in the population of transducing fragments (see ROTH and HARTMAN 1965; and PEARCE and STOCKER 1965), and that *thy* might represent the ends, rather than the middle, of some transducing fragments. In that case, *lys* and *argB* should co-transduce with each other at an unexpectedly low frequency. Table 2 shows that this is so; upon analysis of data from several dozen experiments, joint transduction of *lys* and *argB* was from none to a maximum of 0.5% with the average *ca.* 0.1% (see exception in Table 2). Since *thy-lys* co-transduction is 18–37% and *thy-argB* co-transduction is about the same, one would expect *lys-argB* co-transduction to be considerably higher, if transducing fragments carrying these three loci are homogeneous with regard to the beginning and end of fragments.

Further support for the heterogeneity of transducing fragments in the *thy* region is shown in Table 6, in which the number of *thy*+ transductants obtained is always much lower than with neighboring *lys*+ or *argB*+ markers. Two possible explanations are: (1) *thy* is in a section of the chromosome where crossing over is more difficult, or (2) low transduction numbers represent heterogeneity in donor phage genomes, where *thy*+ fragments are fewer. Of these two possibilities, conjugation tests fail to support the idea that *thy* is in a region of low crossing over, since one obtains as many *thy*+ recombinants as *lys*+ or *argB*+ (Table 3), although it is recognized that the donor genome in conjugation is much longer than in transduction and the two systems of recombination are not identical.

Note that joint transduction of *argB-thy* is always higher when *thy* is the selective marker than when *argB* is the selective marker (Table 6). A possible explanation is that there are more fragments of *argB*-without-*thy* than *thy*-without-*argB*, further supporting explanation (2). If in the donor population the number of particles that carry *thy* is lower than the number that carry *lys* or *argB*, *lys* and *argB* must be closer to *thy* than indicated by the 10–38% joint transduction that is obtained.

It should be noted that similar results were obtained by EGGERTSSON and ADELBERG (1965) with regard to the linked suppressor gene (*sup*-10) and *thy*.

TABLE 6

Differences in joint transduction depending on whether thy *is used as selected or unselected marker. Donor is wild type*

Recipient	Selected marker	Unselected marker	Number of transductions*	% co-transductions
thy341 argB69	*thy*+	*arg*+	179 *arg*+ *thy*+	38%
			294 *arg*− *thy*+	
			442 *arg*+ *thy*+	10%
	arg+	*thy*+	4056 *arg*+ *thy*−	

* Data combined from five Petri plates. Procedure described in DEMEREC, *et al.* (1956). Note that the *number* of *thy*+ transductions (total of 473) is only about 10% of the number of *arg*+ (4498). Experiments in which *thy*+ *lys*− is used as recipient give similar results. The number of transductants of unselected markers was determined by replica plating on appropriate media.

TABLE 7

Differences in joint transduction of thy *and* argB *using different wild-type donor lysates. Recipient was* thy 341 argB 69

Lysate	Selected marker	Unselected marker	% joint transduction
1	*thy*+	*arg*+	38%
	arg+	*thy*+	10%
2	*thy*+	*arg*+	29%
	arg+	*thy*+	12%

Similar to our case, they found that *thy* was transduced less frequently than *sup*. Also, joint transduction was higher if *thy* was the selective marker than if *sup*-10 was the selective marker.

Further evidence of heterogeneity is indicated by the fact that different donor lysates give different values of co-transduciton. It is well known that different lysates will give different ratios of transduction for unlinked markers. It would be expected, however, that if transducing fragments for any set of linked markers were alike, joint transduction of two markers would be independent of the source of the lysate. Table 7 shows that two different donor lysates may give different values of joint transduction, further indication of the heterogeneity of transducing fragments.

ROTH and HARTMAN (1965) were able to demonstrate heterogeneity among transducing particles in the *metE-ivaC* region by differences in densities of phages that carried various markers. In an attempt to do the same, phage prepared on wild-type cells were centrifuged in CsCl, small droplets were collected and analyzed for transducing ability of markers (Figure 2). This experiment was repeated several times, but in no case was it possible to show a difference in peaks between phages that transduce *thy*, *arg*, or *lys*. Therefore, while frequency of co-transduction suggests heterogeneity among fragments that transduce these markers, it was not possible to demonstrate this by any difference in buoyant density between possible *argB-thy* and *lys-thy* transducing particles.

Another case of heterogeneity of transducing particles is provided by PEARCE and STOCKER (1955) involving flagellar and antigenic loci.

Attempts at fine structure mapping of sites within thy *locus:* Numerous transductional crosses of *thy*$^-$ × *thy*$^-$ were made in both two-point and three-point tests, but the numbers of recombinants obtained were always small, and it is felt that the data would not lead to a meaningful genetic map.

Neighbors of thy *locus:* There are interesting neighbors to *thy*. The two loci that straddle *thy* (*lys* and *argB*) control enzymes involved in glutamate pathways. *argB69* may synthesize an excess of lysine or glutamate since it stimulates growth of *lys 6* in crossfeeding (syntropism) tests. *argA*, *argB*, *argC*, *argE* and *argF* mutants did not cause such cross-feeding.

An attempt has been made (DALE, FRIESEN and EISENSTARK, unpublished data) to determine the proximity of the *RC* locus (STENT and BRENNER 1961) to *thy*. This was done by making hybrids of stringent (*RC*+) *S. typhimurium*

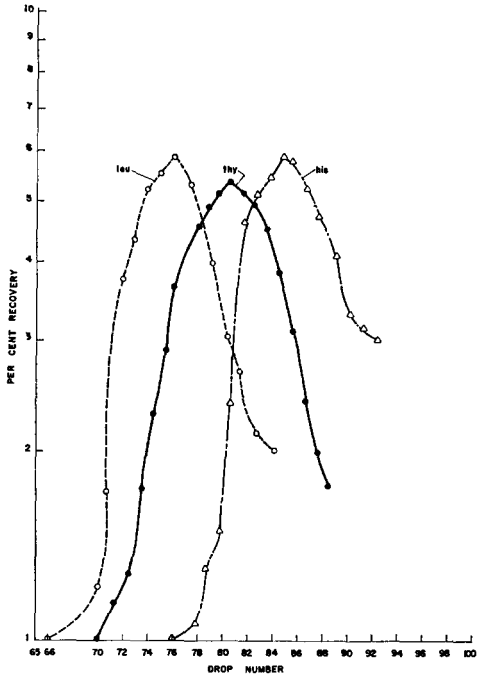

FIGURE 2.—Graph of CsCl density gradient experiment showing difference in density of donor phage particles. The method was that described by ROTH and HARTMAN (1965). Phage P22 was grown on appropriate hosts and concentrated to 10^{12} particles per ml. To each 5 ml centrifuge tube of CsCl (density of 1.500), 0.5 ml of phage samples was added. Centrifugation was at 27,000 for 24 hours in Spinco SW39 rotor. Single drops were collected in 0.5 ml of nutrient broth and assayed for transducing ability using appropriate recipients. Fractions were also assayed for plaque formation. Not indicated on graph are peaks for plaque-forming ability, which gave the same peak as *his*, or for transduction of *arg* and *lys* markers with same peaks as *thy*. *leu* recipient was used for comparison with heavy transducing particles. Points on graph represent averages taken from five separate experiments.

and relaxed (*RC*$^-$) *E. coli* in which analysis showed that the *E. coli* portion in the hybrid genome extended from *lys* to *cys C* (DEMEREC and OHTA 1964); such recombinants were always relaxed (*RC*$^-$). While the exact position of *RC* is still uncertain, transduction tests and tests with episome F15 (which covers *lys*, *thy*, *argB*) indicate that *RC* is not covered by F15, and probably lies between *argB* and *cysC*.

In *E. coli*, other interesting neighbors of *thy* are *mut* (mutator gene) (SEGAL, personal communication), *sup*-10 (suppressor) (EGGERTSSON and ADELBERG 1965), *gal R* (galactose regulator) (BUTTIN 1963), and *rec* (recombinationless) (VAN DE PUTTE *et al.* 1965; EMMERSON and HOWARD-FLANDERS 1967). In *S. typhimurium*, *rec* mutants are currently under investigation, and in at least one case, *rec* lies near *argB* (EISENSTARK, *et al.* 1968).

SUMMARY

Mapping of thymineless (*thy*) mutants of *Salmonella typhimurium* showed that the clockwise order is *argB thy lys* at 91 minutes on the genetic map of SANDERSON and DEMEREC, with close linkage between these markers. The F15 episome covers these three loci but does not extend to include neighboring *serA* or *cysC*. A second *thy* locus was sought but none was found among 80 independent *thy* mutants examined. If there are two or more *thy* loci, they are tightly linked. —A second mutation which allowed cells to grow at lower concentrations of thymine (*tlr*—thymine low requirement) mapped near threonine (*thr*), zero minutes. Data are presented to show that another phenotype, deoxyriboside-sensitivity, is controlled by the same *tlr* locus; deoxyriboside-sensitivity revertants, transductants and conjugal recombinants become tlr^+.—Evidence is presented to indicate that, in a P22 phage lysate, transducing fragments that cover *argB thy lys* are not homologous with regard to their beginnings and ends; i.e., there are many more transducing particles of *argB*-without-*thy* markers than there are *thy*-without-*argB*. However, this heterogeneity of transducing fragments could not be resolved by CsCl density gradient centrifugation of phage lysates.—The relationship of *thy* to neighboring loci is discussed.

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