CYSTEINE MUTANTS OF SALMONELLA TYPHIMURIUM[1]

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NUTRITIONAL and genetical analyses of about 40 cysteine-requiring mutants of *S. typhimurium* have been described by DEMEREC (1955), DEMEREC, BLOMSTRAND and DEMEREC (1955), and CLOWES (1958a,b). These workers concluded that the cysteine mutants represented changes at five genetic loci, designated *cysA, cysB, cysC, cysD,* and *cysE.* The *cysA* locus did not show linkage with other known loci in transduction experiments, nor did *cysE;* the *cysB* locus was linked with a cluster of genes involved in tryptophan biosynthesis (DEMEREC and DEMEREC 1956); *cysC* was transduced simultaneously with *cysD.* In a further analysis of the *cysC–D* region, 134 mutants were studied; these included 30 deletions, 25 of them very similar in extent (DEMEREC 1960). In abortive-transduction experiments, HOWARTH (1958) further divided the original *cysA* region into two complementation units and named these two units loci *cysA* and *cysF.* The number of cysteine-requiring mutants in our collection has increased to about 700, and the analyses have been extended. This paper describes the classification, according to transduction behavior and growth response, of about 425 of these *cys* mutants.

At the time that the earlier studies of cysteine mutants were made, it was considered that a gene locus coincided with a complementation unit, and thus a locus symbol (capital letter) was assigned to each complementation unit as it was detected. There is now ample evidence that a locus may contain several complementation units. Consequently a gene locus can be firmly identified only after completion of a biochemical analysis showing that the unit in question controls the structure of one enzyme. Since complete biochemical analyses of our material are not yet available, we are now calling each independently transducing cysteine unit a "region," but will continue for the present to identify each by a capital letter. The current designations of five *cys* regions are *A, B, C, E,* and *G.*

MATERIALS AND METHODS

The mutants studied were derived from *S. typhimurium* strains LT-2 and LT-7, obtained from DR. N. D. ZINDER. They were isolated by several workers

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in our laboratory, by means of standard penicillin screening after treatment with certain mutagens. The bacteriophage employed in transduction tests was temperate phage PLT-22 H-1 (ZINDER and LEDERBERG 1952).

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, 5.0 g; distilled water, 1000 ml. Enriched minimal medium was made by adding 0.015 percent dehydrated nutrient broth. For plate culture, both media contained 1.5 percent agar. When the media were supplemented with various sulfur compounds, the final concentration per ml was 2×10^{-2} or 2×10^{-4} molar with respect to sulfur (SM).

Nutritional requirements were determined by streaking a suspension of cells on minimal-agar medium containing various sulfur compounds. Syntrophism (feeding) among the mutants was tested by streaking bacterial suspensions at right angles on appropriate media.

In the transduction experiments, recipient bacteria, cultured overnight in cysteine-enriched nutrient broth, were infected with phage grown on the donor bacteria, and plated on suitable medium. The multiplicity of infection was usually about five. Colonies were scored after 24–48 hours of incubation at 37°C.

For scoring abortive transduction, about 6×10^8 cells per plate were spread on enriched minimal medium. Each plate was then divided into five sections, and a drop of a different phage suspension (2×10^{10} phage particles per ml) was placed on each section. One of the two control sections received phage grown on wild-type bacteria, and the other phage grown on the recipient bacteria. After 15–24 hours of incubation at 37°C, abortive transductants were observed under the low-power microscope as minute colonies. Abortive-transduction colonies appeared on the control section with wild-type phage, and on the experimental sections if the mutants involved complemented each other.

RESULTS

Five transducing fragments: In nutritional-requirement tests, made with 2×10^{-4} SM/ml L-cysteine sulfinic acid (CSA) or thiosulfate, 20 mutants were separated into four phenotypic groups. The first group (*cysC-7, -80, -313, -398,* and *-519*) is able to utilize either CSA or thiosulfate. The second group (*cysA-20, -22; cysB-18, -24,* and *-482*) cannot grow on thiosulfate but can grow on minimal medium containing CSA. The requirement of the third group (*cysC-66, -266, -270; cysG-382* and *-439*) is not satisfied by CSA but is satisfied by thiosulfate. The fourth group (*cysB-12, -14, -16; cysE-8, -396*) does not respond to either compound.

These nutritional differences have been utilized to determine whether different *cys* markers are carried in the same transducing fragment. Transduction experiments were performed with recipient bacteria of a strain not able to grow on CSA and a donor strain that does grow on CSA. When such infected bacteria are plated on minimal medium supplemented with CSA, the donor characteristic appears only if the two cysteine markers are located in a region of chromosome carried in one transducing fragment and thus can be jointly transferred.

Transduction tests of this type with about 425 mutants showed that the markers are distributed among five transducing fragments. In other words, five different regions of the Salmonella genome are involved in the control of cysteine biosynthesis. These regions are designated as follows: *cysA* (83 mutants), *cysB* (30 mutants), *cysC* (276), *cysE* (13), and *cysG* (23).

Complementation studies: During the past few years it has been established that one of the best techniques for the determination of a functional unit, or complementation unit, in a bacterial system is abortive transduction (OZEKI 1956; DEMEREC and OZEKI 1959; HARTMAN, HARTMAN and SERMAN 1960). This technique has been used extensively in working out relationships among the cysteine mutants.

Initially about 30 mutants, picked randomly from the five cysteine regions, were examined in all possible combinations for abortive transduction. By the grouping of noncomplementary mutants we established the complementation units for each region; and representatives of each unit were further tested with other mutants of the same region. The results divided 62 single-site mutants of the region *cysA* into three groups: *a*, with 43 mutants; *b*, with eight; and *c*, with 11. Mutants of any one group complemented all members of the other two groups, but not mutants of the same group. Thus, each of the complementation units behaved as a discrete cistron. Two multisite mutants, *cysA-20* and *-533*, failed to complement any of the single-site mutants of this region (*cysAabc*), and the two deletions giving rise to these mutants were found by recombination tests to include all known sites of the *cysA* region.

Twenty-six mutants of the *cysB* region were divided into three groups, *cysBa* (4), *b* (18), and *c* (4). The mutants of each complementation group gave rise to abortive transduction with all mutants of the other groups, but failed to complement all mutants of their own group.

The complementation behavior of 191 single-site mutants of the *cysC* region indicated the existence of five groups: *a* (34), *b* (30), *c* (46), *d* (38), and *e* (43). The complementation pattern of the *cysC* mutants was similar to that of *cysA* and *B* mutants, that is, five discrete cistrons were implicated. Complementation between groups *cysCa* and *cysCb* was weak; under the usual experimental conditions the abortive-transduction colonies were smaller than those appearing in crosses between members of other groups. A similar interaction between mutants of two histidine loci was observed by HARTMAN *et al.* (1960). Two discrete complementation units were observed in the *cysE* region: *cysEa* (four mutants) and *b* (three mutants). Every single-site mutant tested could be placed unambiguously in one of the two groups. Tests with *cysG* mutants have not yet been completed.

Studies with multisite (deletion) mutants showed that they did not complement mutants associated with units wholly or in part covered by the deletion and always complemented mutants belonging to other complementation groups. For example, deletion *cysC-519* includes a portion of the *cysCa* and all of the *cysCb* unit; mutant *cysC-519* does not complement any mutants of groups *a* and *b*, but complements all mutants of the *c*, *d*, and *e* groups. Similarly, all multisite mutants

resulting from deletions covering units *c*, *d*, and *e* complement only mutants belonging to groups *a* and *b*.

Nutritional-requirement and syntrophism tests: The growth responses of 44 cysteine mutants to several sulfur compounds were described by CLOWES (1958a). In our present experiments the nutritional requirements of members of each complementation group are determined by the following procedure. Cells from a culture grown overnight in broth supplemented with cysteine are centrifuged, washed, and resuspended in saline. One loop of the suspension is streaked on minimal medium containing a particular sulfur compound, and the growth responses are scored after 15 to 24 hours of incubation at 37°C.

The cysteine mutants can be classified into four types on the basis of growth responses (Table 1). Single-site mutants of groups *Ca*, *Cb*, and *Ce*, as well as a *Cab* multisite mutant, can utilize sulfite, CSA, or thiosulfate, as sulfur sources. The sulfur requirements of *Cc*, *Cd*, and all *G* mutants can be satisfied by thiosulfate, but not by sulfite. Mutants of the *Aa*, *Ab*, *Ac*, *Ba*, and *Bc* groups, including an *Aabc* deletion mutant, are able to utilize sulfite or CSA, but cannot grow on a 2×10^{-4} SM concentration of thiosulfate. Mutants of group *Bb* and all *E* mutants do not respond to any of these compounds. According to COHN and MONTY (personal communication) H_2S satisfies the requirements of all but *cysE* mutants. The behavior of multisite mutants is the same as that of single-site mutants of the unit affected. If a deletion involves a section of the gene string including more than one unit, the deletion mutant has the more fastidious of the requirements exhibited by the mutants of the affected units. For example, a

TABLE 1

Growth requirements of cysteine mutants (s = single-site; m = multisite). All mutants grow on cysteine, and none grows on $SO_4^=$ salts (sulfate) or L-cysteic acid. Nutritional pattern of L-cysteine sulfinic acid (CSA) and $S_2O_5^=$(meta-bisulfite) is identical with the pattern of $SO_3^=$(sulfite). $S_2O_3^=$ = thiosulfate, $S_2O_4^=$ = dithionite or hyposulfite, $S^=$ = hydrogen sulfide. Concentrations: low = 2×10^{-4}; all others, 2×10^{-2} molar with respect to sulfur (SM)

Region	Mutant	$SO_3^=$	$S_2O_3^=$ Low	$S_2O_3^=$ High	$S_2O_4^=$	$S^=$*
Aa, b, or *c*	s	+	—	+	+	..
Aabc	m	+	—	+	+	+
Ba or *c*	s	+	—	+	+	+
Bb	s	—	—	—	—	+
Ca, b, or *e*	s	+	+	+	+	..
Cc or *d*	s	—	+	+	+	..
C-109, b and *c*	s	—	+	+	+	..
C-519, ab	m	+	+	+	+	+
C-536, ed	m	—	+	+	+	..
C-66, edc	m	—	+	+	+	+
E	s	—	—	—	—	—
G	s	—	+	+	+	+

* Data of E. COHN and K. J. MONTY, The Johns Hopkins University (personal communication).

deletion of the *Ced* section (*Ce* mutants can utilize CSA, *Cd* mutants cannot) produces mutants that cannot utilize CSA.

A 2×10^{-2} SM concentration of thiosulfate or 2×10^{-4} SM of dithionite supports growth of *cysAa, Ab, Ac, cysBa, Bc, cysCa, Cb, Cc, Cd, Ce,* and *cysG* mutants, but not that of *cysBb* or *cysE* mutants. L-cysteine sulfinic acid and metabisulfite can be substituted for sulfite. L-cysteic acid does not support growth of any of the cysteine-requiring mutants tested, nor does cysteic acid serve as a sulfur source for growth of wild-type bacteria (CLOWES 1958a).

Feeding tests made on enriched minimal medium, with incubation for 48 hours at 37°C, showed that *cysCc, Cd,* and *cysG* mutants feed *cysAa, Ab, Ac, Aabc, Ba, Bc, Ca, Cb,* and *Ce* mutants, that is, all mutants that are able to utilize sulfite or CSA. Multisite mutants *cysC-66* and *-536,* although their nutritional requirements are similar to those of single-site *Cc, Cd,* and *G* mutants, do not feed the mutants that are able to utilize sulfite or CSA. Tests on enriched minimal medium supplemented with certain sulfur compounds revealed the following: (1) *Ca, Cb,* and *Ce* mutants feed *Aa, Ab,* and *Ac* mutants on a low concentration of thiosulfate after 60 hours of incubation; (2) *Cc, Cd,* and *G* mutants feed *Bb* and *E* mutants on thiosulfate or methionine; (3) *Aa, Ab, Ac, Aabc, Ca, Cb, Cab,* and *Ce* mutants feed *E* mutants on CSA or methionine.

Order of complementation groups: Mutant sites of the *cysB* region can be mapped by means of three-point crosses, because the transducing fragments that transmit *cysB* also transmit a cluster of four tryptophan loci. In such a cross, if a donor marker brought in by the phage is located between two markers carried by the recipient bacteria, the wild-type recombinant class will result from quadruple crossovers, and consequently will be smaller than the wild-type class that is derived from double crossovers when the donor marker is not located between two recipient markers. Table 2 shows the data from crosses between a *tryD-10 cysBb-12* recipient and nine *cysB* donors. It is evident that the wild-type class is

TABLE 2

Three-point tests with tryD-10 cysBb-12 *as recipient bacteria and* cysBa, Bb, *or* Bc *as donors. Bacteria were plated on enriched minimal medium supplemented with tryptophan and colonies were printed on minimal medium to determine the numbers of wild-type recombinants*

Donor	Complementation unit	No. of colonies on try.	No. of colonies on min.	Percent wild type
cysB-18	a	56	10	18
-24	a	134	22	16
-12	b	0	0	0
-14	b	53	13	25
-87	b	28	5	18
-403	b	45	7	16
-10	c	44	22	50
-15	c	87	39	45
-482	c	38	13	34

considerably larger in crosses with donors of the *c* complementation unit than with those of unit *a* or *b*, an indication that *a* and *b* sites, but not *c* sites, are located between *tryD-10* and *cysBb-12*. Therefore the complementation results are reflected in the chromosome structure by probably three adjacent segments, and the indicated order on the chromosome is: *try—cysBa—cysBb—cysBc.*

Two methods were used in mapping the *cysC* region, namely, two-point crosses and crosses involving overlapping deletions. The nutritional requirements of *cysCa, Cb,* and *Ce* mutants, but not of *Cc* and *Cd* mutants, can be satisfied by CSA. Thus, it is possible to score the donor and recombinant classes in two-point crosses with a *Cc* or *Cd* mutant as recipient and *Ca, Cb,* or *Ce* as donor, when the bacteria are plated on minimal medium supplemented with an amount of CSA inadequate for full growth. On such plates, colonies of the donor class (*Ca, Cb,* or *Ce*) are considerably smaller than those of the wild-type recombinants. The method of overlapping deletions is very reliable for placing mutant sites in definite larger sections of the genetic map. This is accomplished by crossing individual mutants with various deletion mutants and thus locating each mutant site in a portion of the map defined by failure to recombine with a particular series of deletions. The order of sites within each portion is then determined by two-point crosses.

Figure 1 shows the deletions employed in determining the order of the five complementation units, and a few of the single-site markers of each complementation group. From the results of crosses involving these multisite mutations, one may infer that units *a* and *b* at the left end of the map are adjacent to each other, that units *c, d,* and *e* at the right end are adjacent to one another, and that group *d* is between *c* and *e*. No deletion is available extending over the long center section that separates the left (*a, b*) and right (*c, d, e*) sections; therefore we have not been able to determine by the overlapping-deletion method whether the order is (*a-b*)—(*c-d-e*), (*b-a*)—(*c-d-e*), (*a-b*)—(*e-d-c*), or (*b-a*)—(*e-d-c*). Fortunately, however, *dl-519* extends a considerable distance into the left part of the center section, and *dl-392* covers an appreciable portion of the right part, so that the order of the five units can be reliably judged from the recombination

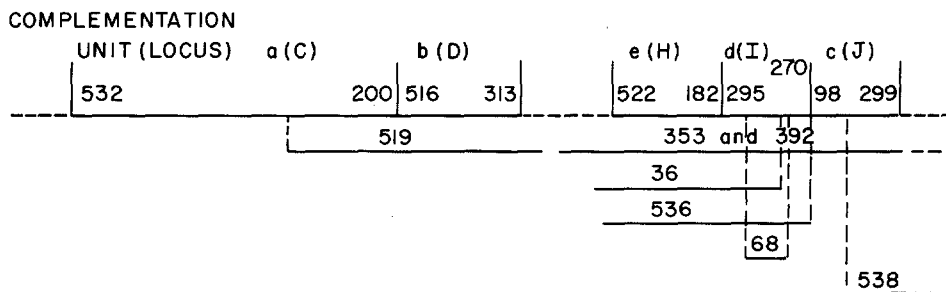

FIGURE 1.—Map of the *cysC* region showing the order of the five complementation units (*a-b-e-d-c*) and the positions of the multisite (deletion) mutations used in determining that order.

frequencies in crosses between *dl-519* and other deletion mutants, and between single-site markers of the *a* and *b* groups and *dl-392*.

The data are presented in Tables 3 and 4. They show (Table 3) that the frequencies of recombination between *dl-519* and six other deletion mutants increase in the following order: *-353*, *-392*, *-36*, *-536*, *-68*, *-538*. Therefore *dl-36* is located closer to *dl-519* than is *dl-536*, and both are closer than either *dl-68* or *dl-538*. The data indicate that the order of the three complementation units is *e-d-c*. Table 4 records the frequencies of recombination between *dl-392* and several single-site markers of the *a* and *b* complementation units. They show that markers included in *dl-519* are as a rule closer to *dl-392* than markers which are not included, and support the conclusion that the order of the two groups is *a-b* rather than *b-a*. Similar results were obtained in crosses between *dl-353* and single-site markers of the *a* and *b* complementation units. The few exceptional data are probably due to excessively high recombination, which is characteristic of particular mutant alleles (DEMEREC, unpublished).

Thus, the indicated order of the five complementation units of the *cysC* region is *a-b-e-d-c*.

DISCUSSION

The results of the transduction experiments show that the sites of mutations leading to cysteine requirement are distributed among at least five different fragments transmitted by phage PLT-22. This evidence alone does not provide adequate information about the location of cysteine-controlling genes in the Salmonella genome. The work of OZEKI (1959) supplied fairly convincing evidence that the fractures which segment transduction fragments are predetermined and therefore that fragments representing any particular portion of the chromosome are similar in extent. Thus two markers located in adjacent sections can be transduced independently, even though they may be very close together. However, the results of conjugation experiments with *Escherichia coli* and with *E. coli-S. typhimurium* hybrids indicate that the cysteine-controlling regions are dispersed along the bacterial genome. It has been known for some time that *cysB* is linked with a cluster of four *try* loci. The linkage map of *E. coli*

TABLE 3

Frequencies of recombination between dl-519 *as donor and six other deletion mutants of the* cysC *region as recipients. Plating was performed on plates partially enriched with CSA upon which* dl-519 *forms small colonies. Both donor* (dl-519) *and recombinant (wild type +) classes were scored*

Recipients	Total	+	Percent +
dl-353	8593	132	1.5
dl-392	9012	144	1.6
dl-36	4593	1826	39.8
dl-536	4675	2484	53.1
dl-68	4282	2573	60.1
dl-538	5921	3776	63.8

TABLE 4

Frequencies of recombination between dl-392 *as recipient and several single-site markers of the* Ca *and* Cb *complementation units as donors. Plating was on CSA-supplemented enriched plates, and both donor and recombinant classes (wild type +) were scored*

Donor	Complementation unit	Total	+	Percent +
Not included in *dl-519:*				
-532	a	692	479	69.2
-537	a	633	374	59.1
-514	a	1294	744	57.5
-428	a	1331	689	51.8
-477	a	816	417	51.1
-520	a	82	40	49.0
-485	a	762	372	48.8
-401	a	1032	481	46.7
-507	a	595	247	41.5
-436	a	1834	618	33.7
-1021	a	695	204	29.3
Included in *dl-519:*				
-405	a	664	284	42.8
-516	b	4269	1581	37.0
-509	a	992	363	36.6
-218	a	614	215	35.0
-80	a	1320	450	34.1
-216	b	1758	593	33.7
-493	b	2947	990	33.6
-78	a	916	299	32.6
-502	b	836	267	31.9
-246	b	354	108	30.5
-200	a	894	273	30.5
-546	b	3426	990	28.9
-135	b	264	74	28.0
-207	b	1131	309	27.3
-378	b	716	182	25.4
-496	b	455	112	24.6
-497	a	4638	1089	23.5
-313	b	2894	656	22.7
-412	b	1066	236	22.1
-505	b	842	183	21.7
-490	b	934	200	21.4
-211	a	1139	240	21.1

prepared by DR. A. L. TAYLOR (1962) places *cysB try* 25 map units from the *thr* locus. In the same map, another cysteine locus (*cys-3*) appears at 58 units, and still another (*cys-8*) at 91. From the results of nutritional tests made by TAYLOR (personal communication) with *cys-3* and *cys-8* of *E. coli*, it seems probable that *cys-3* is homologous with *cysCa, Cb*, or *Ce* of Salmonella, and *cys-8* with *cysA*. Thus three *cys* regions that have been mapped are located far apart on the chromosome.

Complementation studies involving a considerable number of mutants revealed

three complementation units each in regions *cysA* and *cysB*, five in *cysC*, and two in *cysE*. Every single-site mutant tested could be placed in one of these complementation groups; that is to say, each complementation group behaves as a functionally distinct cistron. Mutants failing to complement mutants of adjacent units have not been detected, although such mutants are very frequent among the alleles of many gene loci in Neurospora and of the histidine loci in Salmonella. The results of the complementation experiments raised the following question: Is each complementation unit of a region a separate gene locus, or are the complementation units of a region simply portions of a single locus? To answer this question it is essential to have information about the enzymes controlled by the genetic material of the five cysteine regions. Biochemical studies of these enzymes are being conducted by K. J. MONTY and JACQUES DREYFUSS of the McCollum-Pratt Institute, The Johns Hopkins University. Their results (personal communication) indicate that at least four discrete enzyme activities are separately controlled by the subunits comprising region *cysC*. Thus it is probable that, within region *C*, each complementation unit represents one gene locus. Furthermore, it is possible that each cysteine region containing two or more complementation units represents a cluster of loci.

The wild type in *S. typhimurium*, as well as in many other microorganisms, is able to utilize inorganic sulfate as a sole source of sulfur for growth. Several workers have demonstrated that the biosynthesis of cysteine in various microorganisms proceeds via the reduction of sulfate (PECK 1962). Our genetic analysis indicates that the reduction process is very complex, since a considerable number of gene loci is involved. The number of genes concerned cannot be determined until the enzymological studies are completed. However, if each complementation group represents a gene locus, then at least 14 genes are implicated in the pathway of cysteine biosynthesis.

Among the *cysB* mutants, members of two complementation groups (*a* and *c*) can utilize, CSA, whereas mutants of the third group (*b*) cannot. Since the order of the units is *a-b-c*, the center of the *cysB* region differs from its two ends with respect to CSA utilization. Of the five groups of *cysC* mutants, three (*a*, *b*, and *e*) can utilize CSA and the other two (*c* and *d*) cannot. Units *a* and *b* are adjacent), and separated from units *e*, *d*, and *c* by a long section not carrying any known marker. Mutants of all three *cysA* groups are able to utilize CSA. Presumably, CSA serves as a source of sulfite (LIENWEBER and MONTY 1961). If each complementation unit represents a separate gene locus, eight loci are identified as controlling reactions between sulfate and sulfite. The loci are distributed among three genetic regions. In two of these regions the units involved in the sulfate-to-sulfite steps are separated from each other—in region *cysB* by another locus, and in region *cysC* by a long section of chromosomal material in which genetic markers have not been detected.

According to the system of nomenclature we have adopted (DEMEREC 1956), a mutant character is given a name descriptive of the most detectable effect it produces, and a symbol which is a convenient abbreviation of that name (e.g., *cys* for cysteine requirement, *try* for tryptophan requirement). Similar mutants

of independent origin are distinguished by numbers assigned to them in the order of their isolation (e.g., *cys-1*, *cys-2*). Finally, when the gene locus has been determined, a capital letter designating the locus is included in the mutant symbol (e.g., *tryA-8*, *tryB-2*, *tryC-3*). The locus designation is not essential for identification of a mutant, and it can be changed as the analysis of biochemical blocks progresses.

Cysteine-requiring mutants were among the first to be studied in our laboratory, and the early work showed that several chromosomal areas are involved in cysteine synthesis. The areas were assumed to represent different gene loci, and were called *cysA*, *cysB*, *cysC*, and so forth. More recent work with complementation, however, indicated that some of these areas may be composed of several loci, and we began to call them "regions" instead of "loci." Now that the indications of the genetic studies are being supported by the results of biochemical analysis, we are in a position to begin revising the *cys* designations and assigning capital letters to all identified loci. At present we shall make this assignment only for region *cysC*, partly because it is the best known, both genetically and biochemically, and partly for the practical purpose of facilitating presentation of a genetic analysis of region *cysC* in another paper which is in preparation.

CLOWES (1958b) has recognized two groups of mutants associated with the *cysC* region, one (*cysC*) that utilizes CSA and another (*cysD*) that does not. In our nomenclature we shall retain *C* for the first of the complementation groups (*Ca*), and adopt *D* for the second group (*Cb*), *H* for the third (*Ce*), *I* for the fourth (*Cd*) and *J* for the fifth (*Cc*).

SUMMARY

About 425 cysteine-requiring mutants of *Salmonella typhimurium* have been classified in terms of recombination, complementation, and growth response. Five cysteine-controlling regions have been recognized, namely *cysA*, *B*, *C*, *E*, and *G*. The evidence indicates that they are located in different parts of the genome. Regions *A* and *B* each contain three complementation units, region *C* five units, and region *E* two units. Region *G* has not yet been analyzed. Every tested single-site mutant has been placed unambiguously in one of these complementation groups. This finding, together with the differences in nutritional requirement detected among mutants belonging to different units of the same region (Table 1), raises the question whether or not each complementation unit represents a separate gene locus. Biochemical studies by K. J. MONTY and J. DREYFUSS of The Johns Hopkins University (personal communication) support the affirmative view. If every complementation unit is a different locus, at least 14 gene loci are involved in the control of cysteine biosynthesis. A genetic map of complementation units of the *C* region is presented. On the assumption that these units do coincide with gene loci, the authors propose a new set of locus symbols for the loci comprising the *C* region, namely, *cysC*, *D*, *H*, *I*, and *J*, for the *Ca*, *Cb*, *Ce*, *Cd*, and *Cc* units, respectively.

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