

GENETIC ANALYSIS OF AROMATIC MUTANTS
OF SALMONELLA TYPHIMURIUM

by

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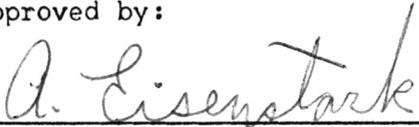
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INTRODUCTION

Following Zinder and Lederberg's (1952) discovery of PLT-22 phage as the vector for the transfer of Salmonella typhimurium genes, transduction has contributed greatly as a tool for analysis of genetic fine structure in Salmonella typhimurium, Escherichia coli, and several other bacteria. Abortive transduction, originally observed by Stocker, Zinder and Lederberg (1953) for the motility character of S. typhimurium, and later by Ozeki (1956) for the adenine-thiamine marker, has been a useful criterion for complementation of nutritional characters in S. typhimurium. Many workers have analyzed bacterial mutants for arrangement of genes along small portions of a linear chromosome map, for relationship between the clustering of genes and functional similarity among the adjacent genes, and for complexity within a gene.

Among auxotroph mutants investigated by transduction and abortive transduction, the majority are satisfied by a single end product (amino acid, purine, pyrimidine or vitamin), while a few single mutants have multiple requirements; among these are adth (adenine and thiamine) (Yura, 1956b), arom (the aromatic compounds, phenylalanine, tyrosine, tryptophan and p-aminobenzoic acid) (Vielmetter, in Demerec et al., 1956) or ilva (isoleucine and valine) (Glanville and Demerec, 1960), each of which can be caused by a single site mutation. These mutants are assumed to block formation of enzymes which are concerned in biosynthesis of these amino acids or vitamins.

The mutants with multiple requirements are particularly useful in studying the complexity of the gene. For example, biochemical analyses

(Wagner and Berquist, 1960) and genetical analyses (Glanville and Demerec, 1960; Armstrong and Wagner, 1964) of ilva mutants have shown the close linkage of three ilva loci which control the biosynthesis of isoleucine and valine in S. typhimurium. Although no genetic fine structure map is available for these ilva or for the adth loci, the latter indicates the presence of complexity within a gene; e.g., by genetic analyses of adth A mutants of S. typhimurium, Yura (1956b) and Ozeki (in Demerec et al., 1956) showed differences among alleles in growth response to pantothenic acid.

Aromatic mutants of E. coli, S. typhimurium and Aerobacter aerogenes were found by Davis and Mingioli (1953) to accumulate two shikimic acid derivatives, Z1 and Z2. This indicated that aromatic pathways of these organisms may be similar. Biochemical studies with E. coli and A. aerogenes mutants by many workers have elucidated the pathway of the aromatic precursors to a great extent, although genetic analysis of these mutants has been meager. Vielmetter (in Demerec et al., 1956) analyzed genetically four tyr, one phe and five arom mutants of S. typhimurium. He found four linkage groups among the arom mutants and designated the loci as follows: arom A-1, arom B-3 and -4, arom C-2, and arom D-5. Since then many mutants have been isolated by several investigators.

In this report arom mutants of S. typhimurium were genetically analyzed and the complex nature of the genes was demonstrated. Although biochemical studies are not available for these arom mutants of S. typhimurium, the genetic analysis has been carried out with the assumption that the aromatic pathway is similar to that of E. coli.

In recognition of the paucity of genetic analyses of arom mutants, this investigation was undertaken, using S. typhimurium as the organism.

REVIEW OF LITERATURE

Allelic mutants of an arom gene are expressed as nutritionally different phenotypes depending on the specific site of the mutation. This was first suggested in the biochemical study of E. coli by Davis (1952a) and is confirmed by the genetic analysis of S. typhimurium in the present report. To understand the nature of this nutritional behavior, it was necessary to examine the literature on genetic organization.

Organization of a gene

Dubinín (1932, 1933) reported cross-overs within an "achaete-scute" gene in Drosophila, and he considered a gene to be divisible by recombination into smaller units; "centers" in his word. Today we know not only that a locus consists of subunits, called sites, but also that a gene is complex and may express itself in various degrees of a phenotype. Whether any two mutations in a gene are "identical alleles" or "non-identical alleles," their phenotype may be distinguishable in many respects (Demerec, 1959).

There are a number of examples of different mutations within the same allele in which there are differences of phenotypic expression. These are reviewed in some detail, since a similar situation was found with regard to the aromatic mutants.

In studies by Giles (1958), allelic mutants of Ad-4 locus in Neurospora showed a difference in the degrees of enzyme (adenylosuccinase) activity as well as in their abilities to undergo reverse mutation in terms of both

frequency and degree of restoration in the enzyme activity. The relationship between the position of a mutational site within a locus and the phenotype of the mutant has also been investigated in the case of adenine-thiamine mutants (adth). Although a precise genetic map is not available, distribution of nine sites within the adth A locus of S. typhimurium indicates that the typical adenine-thiamine phenotype sites are in the central portion of the locus, whereas those representing incomplete biosynthetic blocks are located at the two ends (Ozeki in Demerec et al., 1956).

The tryptophan synthetase locus, try-3, of Neurospora produces a single, inseparable protein and this enzyme protein catalyzes three reactions. It was shown that mutational sites involving different enzymic activities of the protein are clustered at different extremities of the try-3 locus, while those resulting in complete functional deficiency are located in the central region (Bonner, Suyama and DeMoss, 1960).

The hisG gene of Salmonella controls the formation of a pyrophosphorylase which is the first enzyme in the histidine biosynthetic sequence and also inhibited by l-histidine, the end product, or its analogue 2-thiazole alanine. Eight mutations to 2-thiazole alanine resistance were mapped in two distinct regions of the G gene, and the feedback inhibition site and the active site of the pyrophosphorylase are shown to be sufficiently distinct that a mutational alteration at the feedback inhibition site need not affect the function of the active site (Sheppard, 1964). In his study it was also shown that the enzyme from the resistant mutants clustered in one region was more thermolabile than the wild type enzyme and the enzyme from the mutant in the other resistant mutational region. As may

be seen below, interallelic complementation and suppressor phenomena must be involved with specific sites of a gene and with the nature of a mutation, e.g. nonsense, missense, multisite, etc. Because of the necessity to focus attention on the relationship of interallelic complementation and suppression of arom genes to nutritional behaviour, these topics are reviewed in some detail.

Interallelic complementation

When two auxotrophic mutant genes are present in the same cell, restoration of the capacity to grow in the absence of the specific nutrient required by the mutants may occur. This phenomenon is termed complementation. Benzer proposed the term "cistron" for a functional unit as defined by complementation test (Benzer, 1957); each functional unit was presumably concerned with the synthesis of specific polypeptide. Since then, however, the existence of complementation between sites has been demonstrated in many cases; Neurospora crassa td (try-3) locus for tryptophan synthetase (Lacy and Bonner, 1961), ad-4 locus for adenylosuccinase (Woodward, 1958), am locus for glutamic dehydrogenase (Fincham, 1959), pan-2 locus for the conversion of keto-valine to keto-pantoic acid (Case and Giles, 1958), arg-1, -10 and orn-2 (arg-6) loci (Catcheside and Overton, 1958), his-1, -2, -3, -5 loci (Catcheside, 1960), me-2 locus (Murray, 1960), pyr-3 locus (Woodward, 1962), try-1 locus (Ahmad, Khan and Mozmadar, 1964), ad-8 locus for adenylosuccinate synthetase (Ishikawa, 1962), leu-2 locus (Gross, 1962), and in bacteria, hisB and hisD loci of Salmonella (Hartman et al., 1960a,b).

An explanation for this interallelic (or intracistronic or intragenic)

complementation is that it is due to interaction between preformed proteins or polypeptide chains. It was demonstrated in glutamic dehydrogenase of N. crassa that complementation can occur in vitro between purified proteins from two allelic mutants (Fincham, 1962). Since enzymes from interallelic complementation are different from wild type enzymes in regard to heat stability and Michaelis constant for the glutamic dehydrogenase, it is not probable that cytoplasmic products of different alleles undergo some kind of recombination process (Fincham, 1959). Woodward, Partridge and Giles (1958) found that in the ad-4 locus of Neurospora, adenylosuccinase activity of heterokaryons increased when the distance between two alleles increased on the complementation map, although the maximum level of enzyme activity restored was 25% of the wild type. Thus the formation of functional protein by the aggregation of two or more polypeptide subunits offers a plausible explanation of the general facts of interallelic complementation (Hayes, 1964). Although most interallelic complementation maps have been linearly described, some are found in circular form (Gross, 1962; Kapuler and Bernstein, 1963). The Kapuler and Bernstein map suggests that the molecular form of adenylosuccinate synthetase is spiral.

Suppressors

A "suppressor" has been defined as follows: when a mutant reverts to wild type as a result of another mutation at a different locus or site, the second mutation is called a "suppressor" of the first (Hayes, 1964). By definition, reversion by a suppressor mutation leaves the original mutation intact. Since the suppressor phenomenon may result from several different

kinds of mechanism, different explanations have been proposed for each of the cases.

Helinski and Yanofsky (1963) have shown that changes at different sites in a structural gene are capable of restoring catalytic activity to an otherwise enzymatically ineffective protein caused by a mutation, thereby permitting growth on a minimal medium. They used the A gene-A protein system of E. coli tryptophan synthetase. Amino acid sequences of the peptides were shown as follows:

Wild typethr-tyr-leu...//...gly-phe-gly
<u>A46</u> (mutant)thr-tyr-leu...//...glu-phe-gly
<u>A46-PR8</u> (partial revertant)thr-cys-leu...//...glu-phe-gly
<u>PR8</u> (second mutation)thr-cys-leu...//...gly-phe-gly

Also it is interesting that in this case a protein with two amino acid changes is functional, whereas the proteins with either change alone are inactive.

Pardee and Beckwith (1962) have shown that in β -galactosidase mutants (i^-o^0) of E. coli 12, revertants of o^0 have widely different levels of β -galactosidase when analyzed by antigenic activity. These levels are determined by an intra-genic mechanism; namely, a mutable part (the o region) of the structural gene appears to set the rate at which the gene expresses itself. Here o^0 mutations neither map within the repressor sensitive site nor define a site essential for m-RNA transcription (Beckwith, 1964). o^0 mutations, therefore, appear operationally indistinguishable from severe polarity mutations.

Cribbs and Englesberg (1964) found in E. coli that mutations in araB gene affect a coordinated increase or decrease in inducible levels of the

three CRMs (cross reacting materials) of A, B and D.

Ames and Hartman (1963) have shown that in the his operon of Salmonella distribution of polarity mutations was more or less at random and these point mutations not only impair the enzyme activity for the locus but also lower the activities of all of the enzymes made by genes in the operon on one side of the mutated gene. Therefore they suspect that either reading frame mutations (Crick et al., 1961) or nonsense mutations (Benzer and Champe, 1962) produce polarity mutations, and that a rate limiting protein synthesis by minority code of modulator triplet might be suppressed by mutation in the modulator coding s-RNA into normal code. This is analogous to Jacob and Monod's (1961) report on the lac region of E. coli: lac⁺ revertants of a O⁰ mutant produced altered β -galactosidase and they resulted from secondary mutations close to the original O⁰ site. In addition, polarity mutations randomly distributed throughout the Z gene were presumed to reduce transcription of Y and X genes. As for the alkaline phosphatase cistron of E. coli, external suppressors have been reported by Garen and Siddiqi (1962) that are located outside the cistron in which the suppressed mutations occur, and are active only on some of the mutations within the cistron. These suppressible mutations do not produce detectable CRM and show no complementation, whereas majority of the non-suppressible mutations do produce the enzyme protein. The enzyme formed by the suppressed mutants is not detectably different from the normal enzyme in electrophoretic mobility, heat stability or specific activity. This was interpreted as a suppressor which changes nonsense code to sense. The suppressor strain might contain a new kind of t-RNA-AA complex that generates a specific kind of mistake in proteins by substituting one particular amino acid for another. The same

principle is supposed to be working in rII "ambivalent" mutant of T4 phage (Benzer and Champe, 1962) that behaves like nonsense in one bacterial host, but nevertheless makes sense in a second (suppressor containing) host.

Suppressor gene action may in certain cases be concerned with the expression of enzyme activity rather than with enzyme formation. Suskind and Kurek (1959) have shown in tryptophan synthetase gene of Neurospora that a mutant td24 protein is zinc sensitive and there are five non-linked suppressor genes which activate the enzyme by removing the inhibitory metal. Campbell (1961) reported about suppressor sensitive mutants of phage λ that are also pH or temperature sensitive. They produced abnormally sensitive proteins that were stabilized or freed of inhibition by suppressor. Another hypothetical mechanism for suppressor is that it could act by opening up an alternative pathway, although there is no good example of this.

Aromatic amino acid biosynthesis

The pathway of aromatic amino acid biosynthesis in E. coli and A. aerogenes consists of a precursor pathway from DAHP to chorismic acid. From this acid the pathway diverges to formation of amino acids; tyrosine, phenylalanine and tryptophan, also to formation of PABA, PHBA, and 6th factor (Fig. 1). The pathway of the common precursors has been investigated by Salamon and Davis (1953); Weiss, Davis and Mingioli (1953); Davis (1955a); Srinivasan et al. (1956); Srinivasan (1959); Levin and Sprinson (1960); Sprinson (1960); Gibson and Gibson (1962); Rivera and Srinivasan (1962); Gibson and Jackman (1963); Srinivasan, Rothchild and Sprinson (1963).

Mutants defective in the synthesis of aromatic amino acids include

these four nutritional groups: (a) those whose requirement is satisfied by tryptophan (try); (b) those that require tyrosine for growth (tyr); (c) those that require phenylalanine for growth (phe); and (d) those that, by the occurrence of single mutation, require two or more compounds among tyrosine, phenylalanine, tryptophan, p-aminobenzoic acid, p-hydroxybenzoic acid and the sixth factor (arom) (Demerec et al., 1956). The last group arom consists of the mutants which are blocked at the common precursor synthesis pathway. The repeated appearance of a multiple aromatic requirement (arom mutation) would be expected from the common pathway of aromatic biosynthesis, since the occurrence of more than one auxotrophic mutation in a single cell is rare in comparison with the occurrence of a single mutation.

The arom mutants of E. coli consisted of double (tyrosine + phenylalanine) requiring, triple (double + tryptophan) requiring, and quadruple (triple + PABA) requiring mutants (Davis, 1952a). Davis found mutants of these various phenotype at each block in four steps in the successive, common pathway. He postulated the differences in nutritional requirement among the mutants of the same reaction as follows: (a) Differences in the completeness of the genetic block result in different limited rates of synthesis of a precursor, and (b) There is a preferential order in which this common precursor, when present in limited amounts, is converted to its products. The order of the preference is PHBA, PABA, tryptophan and finally phenylalanine and tyrosine.

From the review of literature, it may be seen that Davis pursued the biochemical analysis of arom mutants, but there is now a need to correlate this with fine structure genetic analysis.

MATERIALS AND METHODS

Bacteria and bacteriophage

85 arom, 20 tyr and 14 phe mutants of Salmonella typhimurium were isolated by several investigators from LT2 and LT7 strains after induction by 2-aminopurine, ultraviolet irradiation, diethylsulfate, 5-bromodeoxyuridine, or spontaneously.

Phage stocks were derived from the temperate strain (H1) of PLT22 (Zinder and Lederberg, 1952). Its mutant (H4) was used to obtain non-lysogenic recombinant progeny after transduction. The procedure was to streak each transductant colony for single colony isolation and then to test each progeny clone for its lysogeny. Another mutant (H5) which is normally virulent, but is capable of transducing cells that have been lysogenized with H1 was also used.

E. coli strains were obtained from Dr. B. D. Davis (M83-1, M83-3, M156-53) and from Dr. A. L. Taylor (AB 1359, AB 1320, AB 1321, AB 444). They were used for crossfeeding tests.

Media

The minimal medium contained K_2HPO_4 (10mg/ml), KH_2PO_4 (4.5 mg/ml), $(NH_4)_2SO_4$ (1mg/ml), sodium citrate (0.5mg/ml), $MgSO_4$ (0.05mg/ml) and glucose (4mg/ml). Enriched minimal medium was made by adding 0.01% (single enriched) and 0.02% (double enriched) dehydrated nutrient broth. For plate cultures both media contained 1.5% agar.

Mainly used for growth response were l-tryptophan (TRY), l-tyrosine (TYR), l-phenylalanine (PHE), and p-aminobenzoic acid (PABA). When the medium was supplemented with these compounds, each final concentration was 15µg/ml in the case of amino acids and 1.5µg/ml in the case of vitamins. When spotting each amino acid and vitamin solution onto seeded agar plates, one drop contained ca. 0.03 ml of 2mg/ml and 0.2mg/ml, respectively. The following compounds kindly supplied by Dr. D. B. Sprinson, College of Physicians and Surgeons, Columbia University, are assumed to be the common precursors also for Salmonella: 3-deoxy D-arabino heptulosonic acid 7-phosphate (DAHP), 5-dehydroquinic acid (DHQ), 5-dehydroshikimic acid (DHS), 5-phosphoshikimic acid (PSA) and 3-enolpyruvylshikimate 5-phosphate (EPSP). These chemicals and shikimic acid (SA) were directly placed on a minimal agar plate seeded with a mutant bacterial culture for the growth response test. The arom mutants of Salmonella, however, did not respond to these chemicals. This is possibly because of a permeability barrier.

Feeding test

Intact cells of both strains were streaked near each other on double enriched minimal agar and feeding response was observed after one, two, and three days of incubation at 37 C. When a feeding was positive, a streaked culture showed a heavy growth at an edge of the streak where a feeder strain had been streaked close to it.

In the case of feeding tests between E. coli mutants and S. typhimurium mutants, the latter strains were sonified before use.

Nutritional test

One-tenth ml of each mutant cultured overnight in nutrient broth was spread on a minimal agar plate and one drop of the combined supplement solution was placed on the bacteria. For each mutant, growth around the spots was scored after 24, 48, and 72 hours of incubation at 37 C.

Transduction

Recipient bacteria grown overnight in nutrient broth were infected with phage grown previously on the donor bacteria and plated on a suitable medium. The titer of overnight cultures was about 2×10^9 /ml and 0.1 or 0.2 ml of the culture was spread on a plate. The multiplicity of infection was approximately five. Colonies were scored after 24-48 hours of incubation at 37 C. The technique to detect suppressors by transduction was adapted from that of Yura (1956a). This procedure includes a transduction of a suppressed strain with a phage culture grown on wildtype strain, and then finding the recombinants of prototrophic phenotype which possess a suppressor marker but not an auxotroph marker.

Abortive transduction

For the complementation test, 0.2-0.4 ml of overnight culture was spread on a minimal agar medium plate and a drop of phage suspension (10^{10} to 5×10^{10} /ml) was placed on the seeded bacteria. After 24 hours of incubation at 37 C, abortive transduction was observed under a low power

microscope at 7x to 30x magnification. If the mutants complemented each other (Ozeki, 1956), the minute colonies of abortive transductants were observed. When some strains had too rough a background on the plate for the minute abortive transductants to appear, the cells were centrifuged and resuspended in saline to make 1/10 of the original volume. Plating 0.1 to 0.2 ml of this suspension on a minimal agar successfully reduced the rough growth of background cells in the case of phe mutants where otherwise all the mutants had too rough background.

RESULTS

Genetic analysis of aromatic mutants of S. typhimurium was started at Brookhaven National Laboratory; results of experiments performed there will be summarized in section A of this report. All the successive study performed at Kansas State University will be presented in section B of this report.

Section A

Complementation tests

The aromatic mutants, analyzed by abortive transduction tests, were classified into seven complementation groups. These seven complementation groups were non-linked in transduction by PLT22 phage; the frequency of stable transduction in crosses between mutants in these seven groups was as high as that when they were crossed with wild type. Further evidence for non-linkage of these groups is that no joint transduction was obtained when mutants among these loci were crossed. One of the complementation groups required only phenylalanine, another group only tyrosine. The other five complementation groups included multiple auxotrophs.

Although each complementation group does not necessarily represent one gene, they were tentatively designated as follows: one phe locus (phenylalanine requirement), one tyr locus (tyrosine requirement), five arom loci; A, B, C, D, and E (multiple auxotrophs). These designations are limited since biochemical analyses are not available at present for these

mutants. The designation essentially followed Vielmetter's result (in Demerec et al., 1956), in which arom-1 was in A locus, arom-4 was in B locus, arom-2 was in C locus, and arom-5 was in D locus. One mutant, arom-3 in his report, was in B locus, but in the present report arom-3 was designated as an A mutant, according to the complementation test.

The arom D locus presents a complexity since it seems to consist of several overlapping complementation units (Fig. 2). Since one revertible mutant, aromD-38, does not complement any of the other D mutants, and since there are smaller groups which overlap within the D region, this seems to be an interallelic complementation phenomenon. It may be seen in Fig. 2 that eleven sites are included in one of the subgroups (5), while the other subgroups, (21), (73), (86), (23), (90), (11), (37), and (78), consist of only one or two sites in each.

There is inconsistency between reciprocal tests with D-11, as may be seen in Fig. 2(a). Since one cross showed positive complementation, the negative reciprocal result was ignored to construct the map. Also, D-23 was used only as recipient, since phage with good titer was hard to obtain.

The behavior of mutant D-38 is an interesting one; it is possibly a single site mutation (since it reverts) and does not complement with any of the D mutants. If this is an operator mutant it could influence more than one locus. Therefore the possibility remains that the two complementation groups 37 and 78 are in different loci from the other D mutations. At this stage there is neither a genetic map nor biochemical study available for the D region, and further experiments are left for the future in order to clarify the relation between the D region and its functional property.

Thus complementation tests of aromatic mutants of S. typhimurium show

the possible presence of five arom, one phe and one tyr loci. It was also found that the arom D region has complex complementation groups.

Nutritional property of each complementation group was studied in the following.

Nutritional requirements of arom mutants

Spot tests in which nutrients were placed on agar plates seeded with each mutant culture showed that arom mutants could be classified roughly into four kinds: (1) either tyrosine or phenylalanine alone is required for growth; (2) both tyrosine and phenylalanine are required; (3) tyrosine, phenylalanine and tryptophan are required; and (4) tyrosine, phenylalanine, tryptophan and PABA are required. The results are summarized in Table I. When a mutant requires TRY + TYR + PHE + PABA for good growth but does grow slowly on TRY + TYR + PHE, it was grouped in (4) because it needed PABA to get the maximal rate of growth, as in the wildtype. PHBA did not help growth of the arom mutants with this method, in contrast to the E. coli mutants of Davis' experiment (Davis, 1952a). More sensitive methods might show the effect of this compound. Here we see that each locus has mutants of varying requirements and that the combination of triple and double requirements is always (TRY + TYR + PHE) and (TYR + PHE), respectively. This behavior in arom multiple auxotrophs is consistent with the biochemical study of aromatic mutants of E. coli (Davis, 1952a). No significant correlation was observed between a complementation group of D region and growth requirement of the group.

All of the phe mutants were satisfied by either phenylpyruvic acid or

phenylalanine. Neither tyrosine, p-hydroxyphenylpyruvic acid nor tryptophan can be substituted for them for the growth of phe mutants except that one mutant, phe-14, can grow slightly on tyrosine also.

All of the 16 tyr mutants tested required tyrosine; tryptophan or phenylpyruvic acid could not be substituted, but phenylalanine helps growth of the tyr mutants slightly. Response to p-hydroxyphenylpyruvic acid (the precursor of tyrosine) varies from slight to full depending on the particular tyr mutant.

In summary, it was found that in S. typhimurium each of the five arom loci consists of mutants requiring various number of aromatic compounds for their growth. The mutational blocks, in the pathway of the arom loci were studied by crossfeeding test in the following.

Feeding test

Although there was no feeding response between any two arom mutants, between any two phe mutants nor between any two tyr mutants, arom mutants appeared to be fed by try, tyr and phe mutants. In this experiment phe-3, tyr-3 and tryA-52 were used which could grow on phenylpyruvic acid, tyrosine and anthranilic acid, respectively. When these three mutants were used to feed arom mutants, it was found that (1) growth of all the arom mutants used were stimulated by phe-3, (2) some mutants in (1) were stimulated by tryA-52, (3) some mutants in (2) could be stimulated also by tyr-3 (Table II). Thus phe-3 was the most stimulating feeder among the three, tryA-52 was next, and tyr-3 was the third. It was also seen that there was no arom quadruple requiring mutant which was fed by tyr-3, no arom double mutant which only

phe-3 could feed, and that arom triple requiring mutants had the intermediate character of the two. This result could be explained if we assume that phe-3 accumulated the last common precursor (probably chorismic acid) more than tryA-52 and tyr-3 did, and tryA-52 accumulated the precursor more than tyr-3 did. Thus phe-3 was supplying enough of the precursor for arom quadruple mutants to grow, but tryA-52 and tyr-3 were not. It might be expected further that S. typhimurium cells are permeable for chorismic acid. In contrast five common precursors before chorismic acid in the pathway have shown, when supplied externally, negative response to S. typhimurium arom mutants.

In summary, no feeding response was found among mutants of five arom loci in S. typhimurium, although the mutants of the five arom loci showed growth response to the feeding effects by phe, tyr and try mutants.

Suppressor and partial revertants

Many arom mutants gave partial reversions with less exacting phenotypes for the aromatic amino acid supplements than their original parent mutants. For example, when quadruple requiring mutants were spread on minimal agar supplemented with two or three of the aromatic amino acids, they spontaneously gave rise to revertant-like colonies as well as revertants to wildtype phenotype.

When these revertant-like colonies were tested for their requirements, it was found that they no longer required all of the quadruple supplement; some of them could grow on three, others could grow on two or even one of the aromatic amino acids, phenylalanine or tyrosine. Some of them could

grow on minimal medium although with slower rate than wildtype. The acquired properties of the progeny were in some cases unstable and shifted back to the original parent phenotype after generations, but some progeny gained stability in the new phenotype. Therefore, there was ample reason to suspect changes in their genotype from the original one. The stable, less exacting progeny were studied for the genotype. Table III shows that the less exacting progeny (partial revertants) still possessed the original mutation sites without being reverted to wildtype. The evidence for this was that there were no wildtype recombinants in the transduction between the original mutant and its partial revertant on the selected medium; this medium could support growth of wildtype colonies as well as the partial revertants. It was suspected, therefore, that a secondary mutation must have occurred which changed the original quadruple requirements into a less exacting phenotype.

We may call this secondary mutation a suppressor, if that is not at the same site as the primary mutation. The specificity of the suppressor effect by nine secondary mutations which independently occurred was tested by transduction on eight quadruple mutants representing four arom loci (Table IV). The medium was the minimal agar supplemented with TYR + PHE + TRY. The less exacting strains were used as donor to transduce their suppressor markers into the recipient bacteria of quadruple requirement. When the transductants of less exacting phenotypes appeared, the suppressor effect was considered to be positive. But there is a limitation to this conclusion, since it was not checked further whether or not the less exacting transductants still possessed their original recipient markers besides the transduced suppressor markers. If the transductants had the donor markers

instead of the recipient markers, they must be donor type transductants and not suppressor transductants. Therefore, in Table IV, the bracketed positive signs mean that they are not conclusive.

In the results, specificity of the suppressors to certain locus was observed; A1-1 and A89-1 suppressed only A locus mutants, B49-1, B49-2 and B76-1 suppressed B and C loci mutants, C32-1 and C65-1 suppressed only C locus mutants, D37-1 and D86-1 suppressed only D locus mutants.

Specificity of the suppressors to certain sites was also observed; A89-1 suppressed A89 site but not the A1 site, D37-1 suppressed D37 but not the D86 site, D86-1 suppressed D86 but not the D37 site. The suppressor effects of B49-1, B49-2 and B76-1 is shown in Table XI, in which all the suppressors had similar specificity. In this experiment also, the same limitation as in that of Table IV must be considered for positive signs.

The possibility cannot be excluded, in both the experiments, that some mutational sites were suppressed so effectively that suppressed colonies might not be distinguished from wildtype colonies. The mutation site was investigated for several suppressors in the following and it was found that some of them were linked by transduction with their suppressible mutation sites, although it is not known whether the suppressor sites were located within the arom loci.

In order to locate the second sites, four different partial revertants, A1-1, B49-2, B76-1 and D86-1 were selected to cross with wildtype. These four (as recipient) had background growth light enough to show recombinant colonies on double enriched minimal agar without being hidden by background growth. A1-1 and D86-1 gave rise to transductants when crossed with wild-type, whereas B49-2 and B76-1 did not (Table V). The reason B49-2 and B76-1

did not recombine with wildtype is not known. B49-2 and B76-1 might have had the second site mutations which were not linked by transduction with the original sites and the second site mutant alone could not grow on minimal medium.

The prototrophic transductants that appeared in the cross A1-1 (genotype aromA1⁻su⁻) X phage grown on wildtype bacteria (genotype aromA1⁺su⁺), are expected to have the genotype of aromA1⁺su⁻ or aromA1⁺su⁺; (su⁻ is a suppressor mutation by definition). For this experiment the assumption is required that the su⁻ mutation alone gives growth of near wildtype. When nine of the transductants were used as donors in further transduction with aromA1 (genotype aromA1⁻su⁺), only prototroph recombinants appeared in each cross on medium where suppressed colony (genotype aromA1⁻su⁻) could have grown. Therefore, the su⁻ must have linked with aromA1 site. Similar results were obtained from an experiment with D86-1 suppressor, indicating that the suppressor in D86-1 is linked with the suppressible site aromD86. Thus the secondary mutations in A1-1 and D86-1 are probably linked with their suppressible sites.

There was another class of partial revertants: A1-2, A62-1 and A81-1. It exhibited peculiar behavior in that the suppressor effect of A81-1 was not transduced on A81, as may be seen in Table III. A1-2 and A62-1 did not transduce suppressor to their parent strains either. When A81 and A81-1 were compared as donor in transduction with other mutation sites (A1, A64, and A71), both gave recombinants with similar frequency. The mechanism which hinders transduction of this suppressor effect has not yet been investigated.

The following conclusions are drawn with regard to the suppressor

effect in arom mutants.

- (1) Partial reversions are caused by secondary mutations which changed a phenotype of arom mutants into less exacting characters.
- (2) Since it is not known whether the secondary mutations happened at a distinct place from the primary (arom) mutation sites, there is a limitation in naming the secondary mutations as "suppressor" mutations.
- (3) Each suppressor effect is specific to one of the five arom loci, except that B and C loci seem to be influenced by the same suppressor.
- (4) Some of the secondary mutations are probably linked with their suppressible arom mutations.
- (5) Some of the partial revertants cannot transduce their suppressor effects and some others cannot be transduced by wildtype donor. This indicates that the secondary mutations happened in separate parts from the primary arom mutations.

Section B

Feeding test

Crossfeeding tests did not work between any two arom loci of S. typhimurium, whereas crossfeeding appeared among some aromatic mutants of E. coli strains.

Since the three E. coli strains M83-3, M83-1, and M156-53 are known to block the conversion of DAHP to DHQ, DHQ to DHS and DHS to shikimic acid (SA), respectively (Davis, 1952a)(Fig. 3), they were used as indicators. Sonified arom A, B, C, D, and E mutant cells of S. typhimurium were placed

on the plates seeded with E. coli strains to observe the feeding pattern. The result, shown in Table VI, indicated that all five arom loci of S. typhimurium accumulated DHS or the precursors between DHS and chorismic acid; therefore they were blocked between DHS and chorismic acid in the pathway. Intact S. typhimurium arom mutant cells did not feed E. coli aromatic mutants. E. coli strains used for crossfeeding tests are listed in Table VII. They were tested for requirements by supplementing end products and SA.

Four E. coli aromatic mutants (AB 1320, AB 444, AB 1321 and AB 1359) obtained from Dr. A. L. Taylor were also used as indicators, although the blocks of the mutants were not known. None of them were fed by sonified S. typhimurium mutants. Since the four E. coli strains could respond to crossfeeding among E. coli, as may be seen in Table VIII, they seem to be permeable for the precursors. Table VIII also indicates that a sequence of blocks caused by the four E. coli mutants is; AB 1359 — AB 1321 — AB 444 — AB 1320, in this order along the pathway. From these data the four blocks were tentatively arranged in Fig. 3 with reference to the known (Davis, 1952a) blocks of strains M83-3, M83-1 and M156-53. There are some inconsistencies in Fig. 3; i.e. behaviors of two mutants, M83-1 and AB 1359 are not alike in the feeding response to others, neither are the behaviors of M156-53 and AB 1321. This could not be explained clearly. If the four AB strains of E. coli block as in Fig. 3, all of the five loci of S. typhimurium mutants seem to block the conversion of DHQ or earlier part of the pathway, because there was no feeding of AB 1359 by the S. typhimurium mutants. But this suggests a contradiction to the fact that, as stated above, the five loci of S. typhimurium mutants could feed the E. coli strain

M83-1 which blocks the conversion of DHQ. Thus the crossfeeding tests were not conclusive, although the positive feeding response of M83-1 to S. typhimurium strongly suggested that these mutants were blocked later than DHS.

Suppressor mutation

One mutant stock, aromB-75, was found to give small colonies in addition to wildtype colonies on single enriched minimal agar, when it was transduced as donor with several quadruple requiring mutants of arom B and C loci. Two main questions were: (1) Does this mutant have a second site mutation (suppressor) besides the arom B mutation? (2) If so, where is this second site located, and what is the phenotype when the second site mutation alone is in a cell? The working hypothesis was to assume that the suppressor was a second site and expressed itself as prototroph when alone in a cell. The experiment was done in the following three ways: (1) The stock culture was assumed to have genotype $B75^- su^-$. Transduction $B75^- su^-$ x phage from wildtype ($B75^+ su^+$) gave only prototroph transductants, all of which were expected to have genotype of $B75^+ su^-$ if $B75^-$ and su^- were not linked in a transducing fragment. Out of nine transductants used further as donor in transduction with recipient suppressible mutant $C65^-$, seven had a suppressor effect on $C65^-$, while two did not; the two colonies must have been the product of co-transduction ($B75^- su^- \rightarrow B75^+ su^+$), and the seven were ($B75^- su^- \rightarrow B75^+ su^-$). Therefore $B75^-$ and su^- are separate but linked, and su^- has prototroph phenotype. (2) Six spontaneous revertant prototroph colonies from $B75^- su^-$ were used as donors in transduction with $C65^-$, and all the six

donors gave suppressed colonies in addition to wildtype colonies on C65⁻. Hence the genotype of the revertants were B75⁺su⁻ and not B75⁺su⁺. 3 Two suppressed colonies from the transduction C65⁻ x B75⁻su⁻ phage were used as donor in transduction with C65⁻ and B75⁻su⁻ (recipients). With the C65⁻ no wildtype transductants and only suppressed colonies appeared, while with B75⁻su⁻ many wildtype colonies appeared. Therefore the two donor strains had genotype of C65⁻B75⁺su⁻.

Conclusions of this experiment are: 1) The mutant stock arom B75 has a suppressor mutation at a different but linked region to B75⁻ site. 2) The su⁻ itself is not an auxotrophic, and grows as fast as wildtype. 3) The suppressed colony (e.g., C65⁻su⁻) can grow on minimal medium supplemented with TYR + PHE.

Genetic map of aromA locus

As we have seen in Table I, there is a variety of phenotypes depending on each mutation site in a single arom locus. In the following experiment, a mapping of these phenotypes within a gene was attempted. There was only one multisite mutant available in aromA locus, and none in the other arom loci. This multisite mutant did not give recombinants with some A mutants but did with other A mutants. Also aromA locus contains the largest number of mutants in our stock. Therefore the aromA was chosen for study of mapping within a gene. The following mutants were used for transduction because they were relatively stable mutations.

quadruple requiring: 1, 64, 67, 71, 89, multisite

triple requiring: 42, 70

double requiring: 43, 46, 55, 102

Mutants of the quadruples and the doubles were crossed reciprocally in transduction. The results are shown in Table IX. We see here that the quadruple group (1, 64, 71, 89) and the double group (43, 46, 55) are clearly separate. The double requiring 102 mutation did not show a similar result in reciprocal tests; it might be located between the two groups. The multisite mutation was shown to cover a considerable part of A locus after crossing with a number of mutants located within the multisite region. The edge of the multisite mutation seemed to be located in the quadruple group (1, 64, 71, 89), and the triple group (42, 70) was not covered. It is interesting that this multisite mutant required four aromatic compounds. Further test was done by crossing these non-covered groups with this multisite mutant (Table X). Here it may be seen that the quadruple group locates near the edge of the multisite, the triple group at a farther part, and another quadruple mutation at a still farther part. Although the chromosomal linearity cannot be confirmed from the data of Tables IX and X, the clustering of mutation sites of similar phenotype was observed. The tentative map from these data is shown in Fig. 4. The distances between sites were taken as average transduction frequency shown in Tables IX and X. Average transduction frequencies among sites in a group and sites in adjacent groups were taken as distance on the map.

Thus section B is summarized as follows. It was found that a suppressor mutation could happen at a region distinct from the suppressible arom mutations. Although the effect of suppressor mutations must be considered for the various phenotypes of the arom mutants, the mapping within aromA locus showed the distinct clustering of mutational sites which impair the gene function to a similar extent.

DISCUSSION

Transduction frequency in crosses between mutants in different aromatic loci (including tyr and phe) of S. typhimurium was as high as that with wild type. This suggested that they were not linked in one transducing fragment, in contrast to the cases of the his operon (Ames and Hartman, 1962), try loci (Demerec and Hartman, 1956), leu operon (Margolin, 1963), etc., where several genes of functional similarity cluster in a transducing fragment. If there is any clustering, it may exist in the arom D region where several complementation groups are found and which might consist of more than one locus, or in arom B and C loci where some suppressors influence both the B and C loci but not the other loci. More precise mapping of arom loci of E. coli is now available in conjugation study (Taylor and Thoman, 1964), which shows that the location of four arom loci, one tyr locus and two phe loci, are not clustered. This suggests a loose linkage relationship of aromatic mutant loci of the two organisms.

In the feeding experiment, E. coli strain M156-53 was not stimulated by any E. coli or S. typhimurium mutants which are supposed to be blocked after DHS or shikimic acid. This M156-53 strain did not respond to shikimic acid alone, which seemed to be inconsistent with the expected block position for this strain. Davis (1952b), however, has shown, with the aid of a double mutant blocked both before and after DHS, that this substance competitively interferes with the utilization of its own product, shikimic acid. Thus, in Table VIII, the positive results are only reliable for the ordering of blocks in the pathway. With this in mind, the feeder strains were compared for their ability to feed the others in order to construct the Fig. 3

blocking order. The three E. coli M strains were studied only as to biochemical aspects by Davis (1952a), while the other four E. coli AB strains were studied mainly for their position of genetic loci on chromosomal maps by Taylor and Thoman (1964).

As for S. typhimurium, SA does not penetrate into the cells, which is in contrast to E. coli cells. E. coli cells have been reported to be impermeable for external PSA and Z1 compound, although they can be excreted from the cells (Davis 1955b). Z1-phosphate (EPSP) was later found to be an intermediate, rather than Z1 (Gibson et al., 1962). Since many phosphorylated metabolites cannot penetrate cells (Davis and Mingioli, 1953), PSA and EPSP would have the same difficulty, also. Therefore if the arom mutants of S. typhimurium are blocked at the conversion of DHS to SA or at a later part of the pathway, which was suggested in the text, the failure of the crossfeeding might be explained by the permeability barrier.

In spite of the difference in precursor permeability between E. coli and S. typhimurium, the nutritional requirement in both organisms shows a similar pattern for end product supplements. This may be explained by Davis' work (1952a) on E. coli, in which he suggests the presence of an incomplete block and preferential conversion of chorismic acid. Since many suppressor effects are found to occur as secondary mutations that decrease the degree of exacting phenotype among arom mutant stocks, the following possibilities must be considered as causing the varied requirements of arom mutants: (1) Most arom mutants are single site mutations and the position of each site determines whether the mutant is quadruple, triple, double, or single requiring, (2) alternatively, all of the arom mutations less exacting than quadruple requiring are accompanied by simultaneously induced suppressor

mutations at the other sites, which modify the requirement of the original mutations.

An argument for (1) is: many mutants in our stock were obtained after 2-aminopurine treatment, which induces single site mutation (Freese, 1959), and rarely does it produce double auxotrophic mutants. An argument for (2) is: suppressor mutations occurred spontaneously later as a second mutation to the original quadruple or triple requiring mutants, and they decrease the requirement of the parental strain into triple or double. Also it is not a rare case that some mutants produce suppressor colonies in transduction with the other mutants on minimal medium, which suggests that the suppressor mutations have been induced with relatively high frequency, simultaneously with arom mutations. A wildtype strain does not possess this suppressor effect; therefore at present there is no decisive experiment to choose one of the two cases. However it is plausible that the less exacting phenotype is caused by both the position of the original mutation site and the second mutational site (suppressor).

The suppressor mutation site can be isolated from a double mutant (arom⁻-su⁻) when the suppressor site alone can express itself with distinct phenotype against the original "auxotroph-suppressor" double mutant, as in the case of aromB-75⁻su⁻ strain. For this to be the case, it is also necessary that the arom site and the su site are far enough apart to produce proper frequency of recombination between the two sites. The nature of the suppressor mechanism in arom mutants is not known, since even the linkage relation is not clear between the suppressors and the suppressible sites. One case, su⁻ found in B-75 stock, could indicate that the su⁻ locus is linked with B locus. Three suppressors, which spontaneously occurred as

secondary mutations in B49 and B76 (Table XI), have the same specificity as the su⁻ in B75 stock; therefore, the four suppressors seem to act in a similar way and influence the two loci B and C. The suppressor mutations in A1-1, D86-1 seem to be linked with their suppressible mutations.

If a suppressor is a secondary mutation within a suppressible locus, the su⁻ site might be an up-modulation mutation; i.e., from a modulator triplet to a non-modulator triplet. The mapping of these suppressors will be possible by conjugation technique.

It has been discussed whether the nutritional differences among alleles depend on the position of mutational sites or on the second site mutations. The genetic map of aromA locus was constructed from transduction frequency among alleles, which gives some impression of clustering of similar nutritional sites. This could mean either that the alteration of a phenotype depends on each changed site only, or that different specificities for suppressor are separated in a gene. Further mapping will show more in detail about the gene structure and its functional localization in arom genes.

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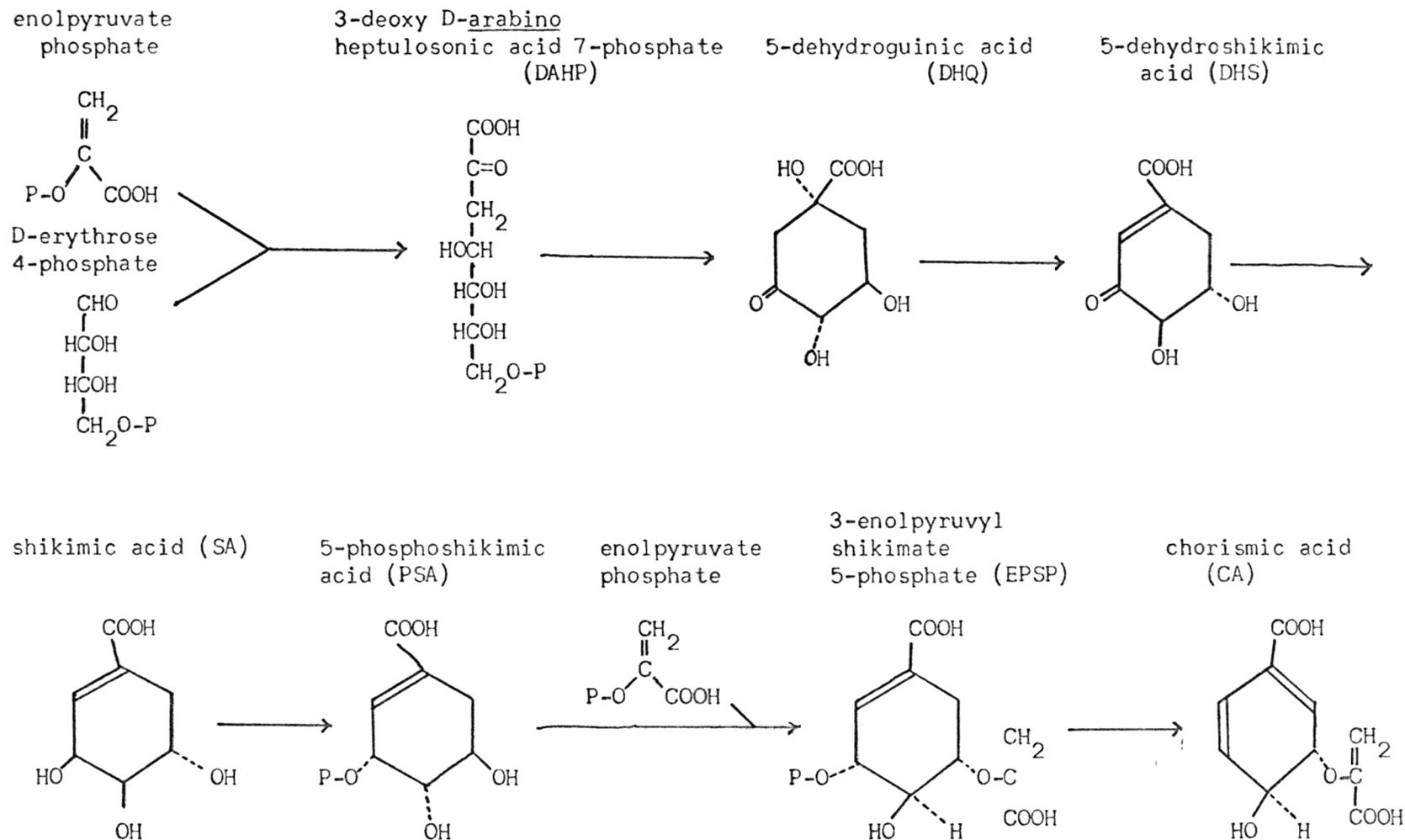
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APPENDIX

FIGURE 1
PATHWAY OF AROMATIC BIOSYNTHESIS*



* Summarized from several biochemical studies with *Escherichia coli* and *Aerobacter aerogenes* (see text).

FIGURE 1 (cont.)

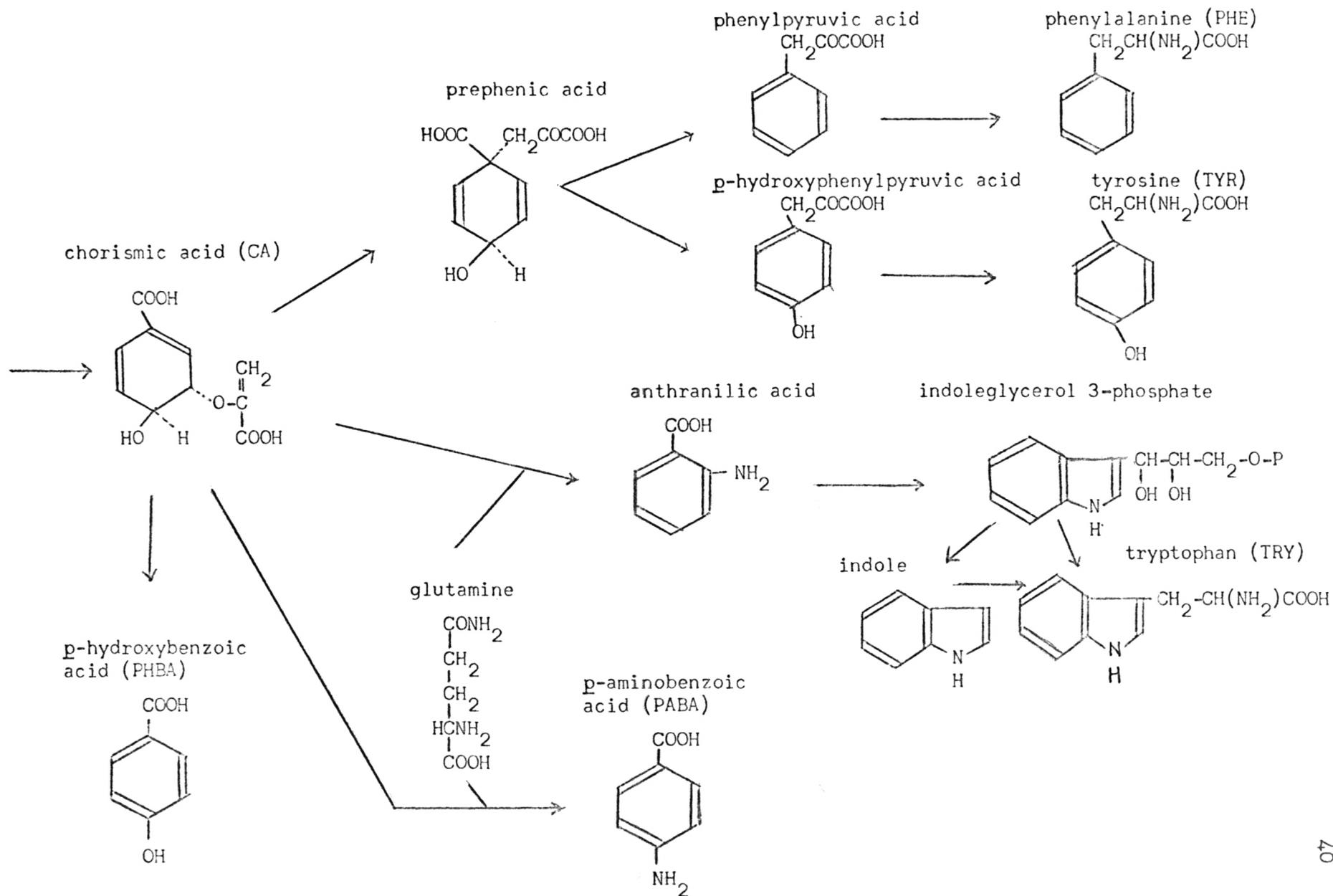


Fig. 2

Complementation map of arom D region. (a) +; positive complementation; -: negative complementation, observed in reciprocal abortive transduction test. (b) lines indicate overlapping ranges of complementation groups where the abortive transduction did not occur on minimal medium and non-overlapping ranges where complementation occurred as indicated by abortive transductants. The length of the lines does not show a distance. (c) The numbers are representative alleles from each complementation group in arom D region.

(a) FIGURE 2
Representative Mutant Number--recipient

D/R	<u>38</u>	<u>21</u>	<u>73</u>	<u>5</u>	<u>86</u>	<u>23</u>	<u>90</u>	<u>11</u>	<u>37</u>	<u>78</u>
<u>38</u>	-	-	-	-	-	-	-	-	-	-
<u>21</u>	-	-	-	+	+	+	+	+	+	+
<u>73</u>	-	-	-	-	+	+	-	-	+	+
<u>5</u>	-	+	-	-	-	-	-	+	+	+
<u>86</u>	-	+	+	-	-	-	-	+	+	+
<u>23</u>										
<u>90</u>	-	+	-	-	-	-	-	-	+	+
<u>11</u>	-	-	-	-	+	-	-	-	+	+
<u>37</u>	-	+	+	+	+	+	+	+	-	+
<u>78</u>	-	+	+	+	+	+	+	+	+	-

(b) Representative Mutant No.

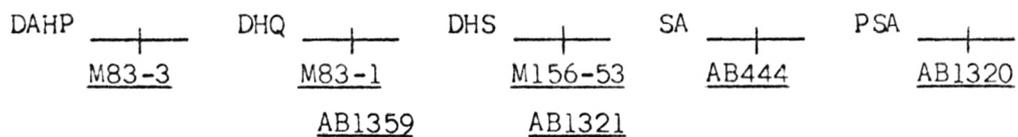
<u>38</u>	_____
<u>21</u>	_____
<u>73</u>	_____
<u>5</u>	_____
<u>86</u>	_____
<u>23</u>	_____
<u>90</u>	_____
<u>11</u>	_____
<u>37</u>	_____
<u>78</u>	_____

(c) Representative No.

Actual Mutant No.

<u>38</u> =	<u>aromD-</u> <u>38</u>
<u>21</u> =	<u>21</u>
<u>73</u> =	<u>73</u>
<u>5</u> =	<u>5</u> , <u>6</u> , <u>17</u> , <u>22</u> , <u>28</u> , <u>35</u> , <u>44</u> , <u>45</u> , <u>57</u> , <u>58</u> , <u>80</u>
<u>86</u> =	<u>86</u>
<u>23</u> =	<u>23</u>
<u>90</u> =	<u>90</u>
<u>11</u> =	<u>11</u>
<u>37</u> =	<u>37</u> , <u>77</u>
<u>78</u> =	<u>78</u>

FIGURE 3

MUTATIONAL BLOCKS IN AROMATIC PATHWAY OF E. COLI

Blocks of M83-3, M83-1, and M156-53 are known from the data of Davis (1952a). They were used as references to investigate the blocks of the strains AB1359, AB1321, AB444, AB1320.

FIGURE 4

GENETIC MAP OF AROM A LOCUS

The distance number is the percentage of transduction between two markers. The experiments are in Table IX and X.

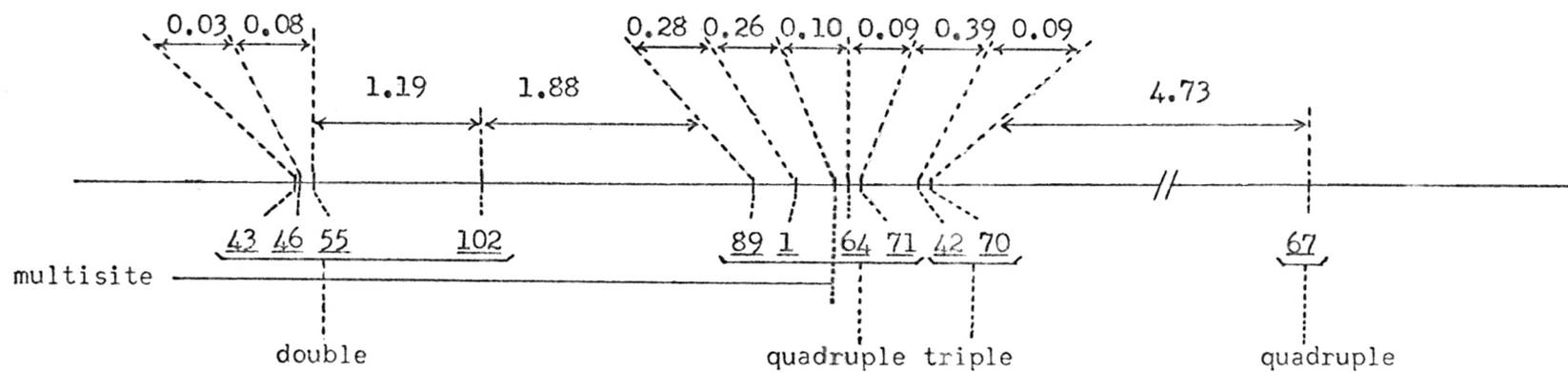


TABLE I

Nutritional requirements as determined by spotting the supplements on a seeded agar medium and observed after three days incubation at 37 C.

Locus	Classification	Total no. of mutants	Requirements for growth	Allele no. of mutants
<u>arom A</u>	Quadruple	9	TRY+TYR+PHE+PABA	<u>1, 15, 20, 40, 62, 64, 67, 71, 89</u>
	Triple	14	TRY+TYR+PHE	<u>9, 10, 13, 18, 19, 42, 53, 70, 72, 81, 84, 95, 101, 201</u>
	Double	11	TYR+PHE	<u>3, 16, 29, 43, 46, 87, 91, 97, 98, 102, 103</u>
	Single	<u>1</u> <u>2</u>	PHE PHE or TYR	<u>48</u> <u>54, 55</u>
	Total	37		
<u>arom B</u>	Quadruple	4	TRY+TYR+PHE+PABA	<u>34, 49, 50, 51, 66, 74, 76</u>
	Triple	6	TRY+TYR+PHE	<u>8, 25, 33, 39, 47, 75</u>
	Double	1	TYR+PHE	<u>12</u>
	Single	<u>0</u>		
Total	14			
<u>arom C</u>	Quadruple	4	TRY+TYR+PHE+PABA	<u>32, 36, 65, 79</u>
	Triple	2	TRY+TYR+PHE	<u>2, 26, 52</u>
	Double	0	TYR+PHE	
	Single	<u>0</u>		
Total	6			

TABLE I (cont.)

Locus	Classification	Total no. of mutants	Requirements for growth	Allele no. of mutants
<u>arom D</u>	Quadruple	6	TRY+TYR+PHE+PABA	<u>21, 35, 37, 58,</u> <u>73, 86</u>
	Triple	15	TRY+TYR+PHE	<u>6, 11, 17, 22,</u> <u>23, 28, 38, 44,</u> <u>45, 57, 77, 78,</u> <u>90, 96, 100</u>
	Double	2	TYR+PHE	<u>5, 80</u>
	Single	<u>0</u>		
	Total	23		
<u>arom E</u>	Quadruple	2	TRY+TYR+PHE+PABA	<u>41, 68</u>
	Triple	<u>2</u>	TRY+TYR+PHE	<u>85, 88</u>
	Total	4		

TABLE II

Number of arom mutants fed by tryA-52, phe-3, and try-3.

<u>Arom</u> mutants used as recipients in feeding test	<u>Feeder</u>		
	Only <u>phe-3</u> can feed	Either <u>phe-3</u> or <u>tryA-52</u> can feed	Either <u>phe-3</u> , <u>tryA-52</u> , or <u>try-3</u> can feed
<u>arom A</u>			
Quadruples	4	0	0
Triples	10	1	0
Doubles or Less	0	7	3
<u>arom B</u>			
Quadruples	6	1	0
Triples	0	2	3
Doubles or Less	0	0	2
<u>arom D</u>			
Quadruples	2	0	0
Triples	5	5	0
Doubles or Less	0	2	4
<u>Total</u>			
Quadruples	12	1	0
Triples	15	8	3
Doubles or Less	0	9	9

TABLE III

List of partial revertants. 1st and 2nd columns designate original arom mutants. 3rd and 4th columns designate partial revertants derived from the originals (e.g. A1-2 derived from A1). 5th and 6th columns show transduction between the original mutant (recipient) and its partial revertant (donor).

Parents		Partial Revertants		On Selected Medium, Parent Cells x Phage Grown on Revertant	
Mutant	Requirement	Mutant	Requirement	Recombinants (revertant phenotype)	Recombinants (wild-type phenotype)
<u>aromA1</u>	quadruple	<u>A1-1</u>	double	500**	0***
		<u>A1-2</u>	triple	0	0
<u>A62</u>	quadruple	<u>A62-1</u>	double	0	0
<u>A81</u>	triple	<u>A81-1</u>	close to protrophic	0	0
<u>A89</u>	quadruple	<u>A89-1</u>	double	58	0
<u>B49</u>	quadruple	<u>B49-1</u>	double	4,000	0
		<u>B49-2</u>	double	3,000	4*
<u>B76</u>	quadruple	<u>B76-1</u>	double	100	0
<u>C32</u>	quadruple	<u>C32-1</u>	phenylalanine or tyrosine	200	0
<u>C65</u>	quadruple	<u>C64-1</u>	double	120	0
<u>D37</u>	quadruple	<u>D37-1</u>	double	100	0
<u>D86</u>	quadruple	<u>D86-1</u>	double	300	1*

*Spontaneous revertants

**This column shows the number of donor type transductants

***If the donor (e.g. A1-1) had the wildtype genotype (A1⁺), this column would have shown many wildtype recombinants.

TABLE IV

Transduction of suppressor phenotype to recipient quadruple requiring mutants.

Phage Grown on	Recipient Bacteria							
	<u>A1</u>	<u>A89</u>	<u>B49</u>	<u>B76</u>	<u>C32</u>	<u>C65</u>	<u>D37</u>	<u>D86</u>
<u>A1-1</u>	+	(+)	-	-	-	-	-	-
<u>A89-1</u>	-	+	-	-	-	-	-	-
<u>B49-1</u>	-	-	+	(+)	(+)	(+)	-	-
<u>B49-2</u>	-	-	+	(+)	(+)	(+)	-	-
<u>B76-1</u>	-	-	(+)	+	(+)	(+)	-	-
<u>C32-1</u>	-	-	-	-	+	(+)	-	-
<u>C65-1</u>	-	-	-	-	(+)	+	-	-
<u>D37-1</u>	-	-	-	-	-	-	+	-
<u>D86-1</u>	-	-	-	-	-	-	-	+

+ Suppressor phenotype appeared as recombinants in the cross.

(+) Not conclusive.

- No suppressor effect was found in the cross.

Selected medium for suppressor effect contained TRY+TYR+PHE.

TABLE V

Transduction of partial revertants with H_4 phage grown on wildtype bacteria. This table shows that the two partial revertants, B49-2 and B76-1, have lost the ability to be transduced to prototroph by wildtype donor, whereas the other three, A1-1, A1-2 and B86-1, maintain the ability.

Recipient Bacteria	:	Recombinants/ 2×10^8 cells
<u>A1-1</u>		100
<u>A1-2</u>		49
<u>B49-2</u>		0
<u>B76-1</u>		0
<u>B86-1</u>		70

TABLE VI

Crossfeeding of E. coli by S. typhimurium (aromatic mutants).*

<u>E. coli</u>	<u>S. typhimurium</u>				
	<u>arom A1</u>	<u>B49</u>	<u>C65</u>	<u>D86</u>	<u>E88</u>
<u>83-3</u>	+	-	+++	+	++
<u>83-1</u>	+++	+++	+++	+++	+++
<u>156- 53</u>	-	-	-	-	-

* The latter was sonicated and placed on seeded E. coli mutants on minimal agar. +, positive feeding; -, no feeding.

TABLE VII

Growth requirement of E. coli aromatic mutants.

Strain no.	Requirements for end products tested	Growth by precursor
<u>M83-3</u>	TRY + TYR + PHE + PABA	*SA, DHS (for total req.) DHQ (for part of req.)
<u>M83-1</u>	TRY + TYR + PHE + PABA	*SA, DHS (for total req.)
<u>M156-53</u>	TRY + TYR + PHE	*SA + TYR + PHE
<u>AB1359</u> (<u>aromD</u>)	TRY + TYR + PHE + PABA	Slightly stimulated on SA
<u>AB1320</u> (<u>aromB</u>)	TYR + PHE	Do not grow on SA
<u>AB1321</u> (<u>aromA</u>)	TRY + TYR + PHE + PABA	"
<u>AB444</u> (<u>aromC</u>)	TYR + PHE	"

*, *, * Data cited from Davis (1952a); growth by SA was confirmed by author.

Requirements for end product for the growth of M83-3, M83-1 and M156-53 are quintuple (TRY + TYR + PHE + PABA + PHBA) in Davis (1952a). Thus the assaying method may have influenced the results.

TABLE VIII

Crossfeeding among E. coli aromatic mutants.

Fed	Feeder						
	: <u>AB1320</u>	: <u>AB444</u>	: <u>M156-53</u>	: <u>AB1321</u>	: <u>AB1359</u>	: <u>M83-1</u>	: <u>M83-3</u>
<u>AB1320</u>	-	-	-	-	-	-	-
<u>AB444</u>	+	-	-	-	-	-	-
<u>M156-53</u>	-	-	-	-	-	-	-
<u>AB1321</u>	+	-	-	-	-	-	-
<u>AB1359</u>	+	+	-	-	-	-	-
<u>M83-1</u>	+	+	+	+	-	-	+*
<u>M83-3</u>	+	+	+	+	+	+	-

* May be explained by feedback.

Mutants were streaked on minimal agar close to each other. +, positive response; -, no response.

TABLE IX

Percentages of transduction among arom A mutations. The donors were compared with the wildtype donor (as 100%) for their transduction frequency with each recipient strain.

Mutant No. : of Donor	Mutant No. of Recipient							
	<u>1</u>	<u>64</u>	<u>71</u>	<u>89</u>	<u>43</u>	<u>46</u>	<u>55</u>	<u>102</u>
<u>1</u>	0.00	0.48	0.81	0.56	15.4	1.92	7.87	4.16
<u>64</u>	0.04	0.00	0.00	0.64	4.2	1.86	3.92	2.58
<u>71</u>	0.32	0.00	0.00	0.96	14.1	4.77	3.83	3.00
<u>89</u>	0.00	0.64	0.50	0.00	55.4	8.67	11.9	6.65
<u>43</u>	1.30	3.03	1.24	4.41	0.16	0.00	0.00	2.46
<u>46</u>	1.11	6.68	3.72	4.06	0.04	0.00	0.08	4.64
<u>55</u>	1.25	3.21	3.08	4.02	0.36	0.07	0.00	2.63
<u>102</u>	0.25	1.19	0.21	0.92	3.38	1.03	1.05	0.00
Wild Type	100	100	100	100	100	100	100	100

Transducing potencies of each phage culture were adjusted by crossing with non-linked recipient aromB-74. For each crossing, 2×10^9 recipient cells were used.

TABLE X

Percentages of transduction among arom A mutants.

I; the multisite mutant was used as donor, and the mutants of the first column were used as recipient. The wild type was used as donor.

II; the multisite mutant was used as recipient, and the mutants of the first column were used as donor. The wild type was used as donor.

The number of transductants between a donor marker and a recipient marker was compared with the number of transductants between the wild type and the recipient marker, then the ratio was shown in percentage. The last column shows the average percentage of the two experiments.

Mutant No.	I Multisite as doner	II Multisite as recipient	Average of I and II
<u>1</u>	0.00	0.00	0.00
<u>64</u>	0.00	0.19	0.10
<u>71</u>	0.15	0.22	0.19
<u>89</u>	0.00	0.00	0.00
<u>42</u>	0.67	0.49	0.58
<u>70</u>	1.09	0.25	0.67
<u>67</u>	5.01	5.78	5.40
wild type	100.00	100.00	100.00

Transducing potencies of each phage culture were adjusted by crossing with non-linked recipient aromC65. For each crossing, 2×10^7 recipient cells were used.

TABLE XI

Specificity of suppressors in B49-1, B49-2 and B76-1.

Phage grown on	Recipient bacteria for transduction									
	<u>B34</u>	<u>B49</u>	<u>B50</u>	<u>B51</u>	<u>B66</u>	<u>B74</u>	<u>B76</u>	<u>C32</u>	<u>C65</u>	<u>C79</u>
<u>B49-1</u>	-	+	-	-	(+)	-	(+)	+	(+)	-
<u>B49-2</u>	-	+	-	-	(+)	-	(+)	(+)	(+)	-
<u>B76-1</u>	-	(+)	-	-	(+)	-	+	(+)	(+)	-

+ Suppressed, (+) not conclusive, - No effect of suppressor

Selected medium for suppressor effect contains TRY + TYR + PHE.

GENETIC ANALYSIS OF AROMATIC MUTANTS
OF SALMONELLA TYPHIMURIUM

by

YASUO NISHIOKA

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AN ABSTRACT OF A MASTER'S THESIS

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1965

Salmonella typhimurium mutants requiring aromatic compounds were investigated genetically.

Complete and abortive transduction tests showed the presence of at least seven non-linked loci; five loci for multiple requirement (arom), plus one locus for phenylalanine (phe), plus another locus for tyrosine (tyr) requirement. The five arom loci were named arom A, B, C, D, and E. Arom D region consisted of several complementation groups, which appeared to be a phenomenon of interallelic complementation. The possibility was also discussed for the presence of more than one gene in the arom D region.

The nutritional behavior of arom mutants were complex within each complementation group; the requirement varied depending on each mutation, from quadruple (tryptophan + tyrosine + phenylalanine + p-aminobenzoic acid), to triple (tryptophan + tyrosine + phenylalanine), to double (tyrosine + phenylalanine), and to single (tyrosine or phenylalanine). This is analogous to Escherichia coli aromatic mutants investigated biochemically by Davis, who had also explained this phenomenon to be caused by incomplete block of mutations and preferential conversion of the last common precursor. The mapping of arom A locus, using several A mutants of the various phenotypes, indicated that the mutational sites of each phenotype (quadruple, triple or double requiring) are not arranged at random but tend to cluster within the A locus. This strongly suggested that the A gene function was impaired to various extent depending on the portion altered by each mutation. Thus, severe mutational damages would cause quadruple requiring phenotype, whereas mutational damages at less important portion of the enzyme would cause more leaky (therefore less exacting) phenotypes.

It was also shown that the quadruple requiring phenotype could be changed into that of triple, double, single or near prototrophic requiring by secondary mutations which took place later in the same cell. Although these secondary mutations could not be located in their positions, some of them seemed to be linked with their suppressible mutation sites. All the suppressor effects tested showed their specificity as to locus, within which they were also specific as to site. Thus the phenotype of an arom mutant seemed to be determined both by the mutational site within a gene and by the presence of a secondary mutation (suppressor).

Crossfeeding tests indicated that the mutational blocks of five arom loci might be at the conversion of 5-dehydroshikimic acid to shikimic acid or at the later steps along the precursor pathway. Thus, the nutrition tests and crossfeeding tests suggested that the aromatic biosynthesis in E. coli and S. typhimurium follows a similar pathway.