H. H. PLOUGH
Amherst College

Chapter 17

*Genetic Implications of Mutations in S. Typhimurium**

The contribution that an account of studies in bacterial genetics can make to the problem of heterosis must be indirect, since actual sexual or other fusion in bacteria has not been observed and the weight of evidence is against the view. Even the very interesting genetic evidence of recombination discovered by Tatum and Lederberg (1947) in the K12 strain of the colon bacillus, and now being developed by the capable studies of Lederberg (1947, 1949) and others, is still susceptible of other interpretations. Diploid strains, if they occur at all, are certainly so rare as to be unimportant in the production of hybrid vigor in bacterial populations.

The applications of bacterial genetics to the problem of heterosis must be rather in the information they make available concerning the kinds and frequencies of gene mutations, and the ways in which they interact with each other within populations. It has been generally recognized by geneticists only recently that the bacteria are excellent material for studies of these problems, though bacterial mutation was first mentioned by Massini in 1907, and distinctive and precise food requirements for bacterial strains have been known since 1913 (Hinselwood, 1946). Studies in the genetics of bacteria have, of course, been greatly stimulated by the pioneer work on mutations in fungi by Thom and Steinberg (1939), and particularly on Neurospora by Dodge, by Lindegren, and by Beadle (1949) and his associates, as well as by the important work on yeast as presented in Dr. Lindegren's chapter. Long before the currently enlarging wave of interest in bacteria as objects of genetic study, Gowen had shown that mutations of the same order of frequency as in higher plants or animals were induced by radiation in Phyto-

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monas (1945). He and Zelle had indicated the genetic basis of virulence in Salmonella (Zelle, 1942).

ADVANTAGES OF SALMONELLA FOR GENETIC STUDIES

I became acquainted with the Enterobacteriaceae and particularly with the pathogenic forms in Zinsser's laboratory at the Columbia Medical School. My own realization that Salmonella offered excellent material for studies in microbial genetics was heightened when, as an Army bacteriologist in the Philippines, I had to diagnose enteric infections. I found most of the Salmonellas which Flexner first described from Manila still present in the islands. More than 140 strains or species of Salmonella are recognized which are distinguishable by a common pattern of fermentation reactions (dextrose and maltose-AG, lactose and sucrose-negative, citrate and H_2S positive). Each one has been shown by the serological studies of White (1929), Kaufmann (1944), or Edwards and Bruner (1942) to have a very precise and readily separable antigenic constitution.

The antigens are determined by agglutination studies using serums from different rabbits immunized to one or another of the major strains. They fall into two distinct groups: the somatic (O) antigens associated with the surface protein layers, and the flagellar (H) antigens determined by proteins of the flagella. Each of these groups is known to be compound, with some twenty separate O antigens—each strain may carry three or four (O) antigens—and eight or ten different specific (H) antigens as well as certain alternative and non-specific phases of the latter. Thus each strain can be shown to have a distinctive and readily determinable antigenic constitution (*S. typhimurium* is I, IV, V, XII—i, 1, 2, 3). The whole group naturally falls into a tree-like pattern very like the evolutionary trees made for families of animals or plants on the basis of structure.

Tatum's (1946) discovery that mutagenic agents (including radiation and nitrogen mustards) could induce mutants of colon bacteria having constant growth factor requirements more limited than the parental organism, just as with Neurospora, has re-emphasized the one gene–one enzyme hypothesis. It has strengthened the idea of bacterial evolution developed by Lwoff (1943) that the parasitic forms have been derived from the less exacting heterotrophic organisms by successive losses of synthetic abilities. Thus it gives added meaning to the tree-like interrelationships suggested by the antigenic analyses.

Soon after the war our Amherst group entered on an intensive study of induced biochemical and antigenic mutations in the food poisoning organism, *Salmonella typhimurium*. It was our hope that this organism would prove more favorable for genetic studies than *E. coli*, not only for the analysis of the mode of action of genes, but for evidence on the genetic nature of type specificity, virulence, and their bearing on evolutionary relationships.

METHODS OF INDUCING AUXOTROPHIC MUTATIONS

The strains of *Salmonella typhimurium* which we have used are two: 519 received from the New York Salmonella center at Beth Israel Hospital, and 533 (11c) from Gowen.

Our method for isolating mutations to specific food or growth factor requirements by penicillin screening is that of Lederberg and Zinder (1948) and of Davis (1949) with some additions of our own. *S. typhimurium* is a heterotrophic organism of the least exacting sort. Cultures will grow on a basic medium containing ammonium sulphate, sodium chloride, potassium phosphate buffers, with traces of other metallic ions, and glucose added as an energy source. Better growth is obtained with a supplementary nitrogen source, such as asparagin, and a further energy source, citrate, but these are not essential. Thus the organism synthesizes all its own food components, coenzymes, and growth factors, as well as the enzymes necessary for food and energy tranformations.

Suspensions are subjected to radiation by X-rays (up to 100,000 roentgens) or ultraviolet light (up to 3,600 ergs per $mm.^2$), and are then transferred to an enriched nutrient broth for 24 hours. The broth stimulates active division of all organisms. These are centrifuged off, washed, and reinoculated for 24 hours into the basic or minimal medium containing 100 units per ml. of penicillin. This stops the divisions, and progressively kills the organisms which divide actively.

These organisms which penicillin screens out are called *prototrophic* (Lederberg), and they are, of course, the unchanged originals. Any mutated organisms which now require some specific nutrilite will not divide on the basic medium, and so they are not affected by penicillin. These are now *auxotrophic* organisms (Davis), and they are isolated by plating on complete agar, and identified by paper disc inoculations on successive plates of basic medium with single nutrilites added—amino acids, nucleic acid fractions, or vitamins, as shown in Figures 17.1 and 17.2. These methods are described in more detail by Plough, Young, and Grimm (1950).

AUXOTROPHIC MUTATIONS FROM RADIATED LINES

I shall cite only one set of isolations from such a radiation experiment, the data for which are given in Table 17.1. Suspensions from an unradiated control and from seven successively increased X-radiation dosages were run through the penicillin screening, and 500 auxotrophic mutants isolated. Of these a total of 459 were recovered and their specific requirements determined. Although the control had been derived from successive single colony isolations within 3 days of the tests, still 5 per cent of the isolated strains were mutants—indicating that spontaneous mutation occurs and accumulates in stock strains.

From the major strain used (#533), 234 strains out of the 459 isolated

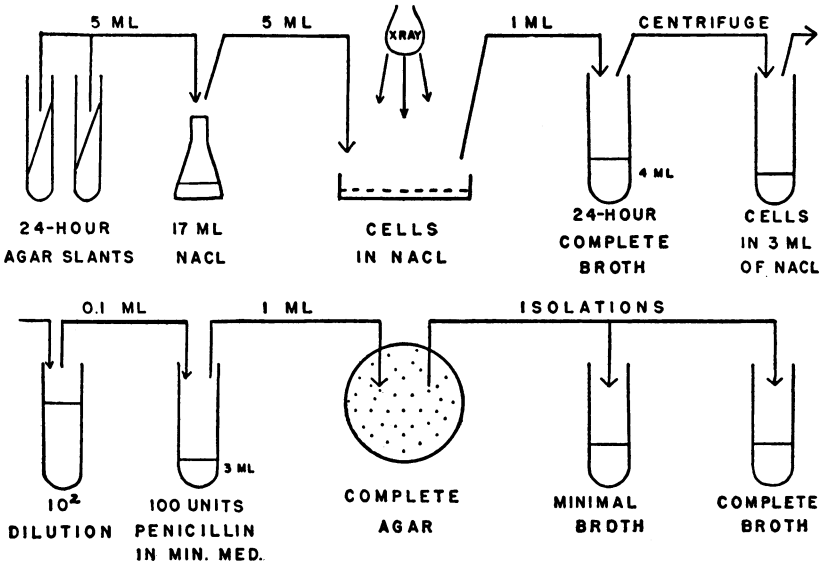

FIG. 17.1—Diagram showing methods for the production of radiation-induced auxotrophic mutations in Salmonella and for their isolation by screening through minimal medium containing penicillin.

PAPER DISC METHOD FOR TESTING BIOCHEMICAL MUTANTS

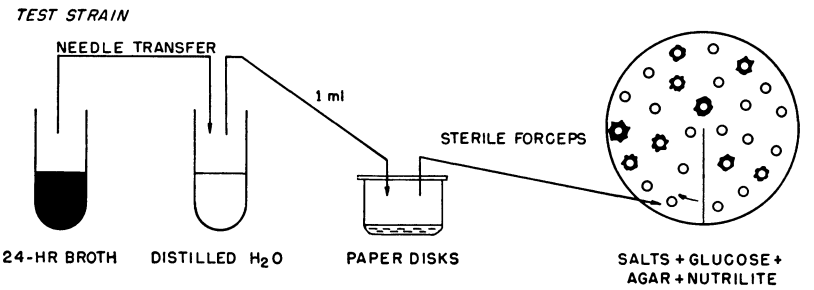

FIG. 17.2—Diagram showing method for determining the particular nutrilite required by the auxotrophs isolated as in Figure 17.1. A series of Petri plates is used, each containing a different test substance.

were auxotrophic mutants, among which 17 different auxotrophic mutants occur according to tests of the specific nutrilite required. A summary of these requirements with the numbers of each is given in Table 17.2. The most frequent auxotroph is the one requiring cysteine. The next most frequent is the histidine auxotroph, and so on down the list to one which has a double requirement of both valine and isoleucine for growth. Only two auxotrophic mutants require substances other than amino acids. One must be supplied

TABLE 17.1

FREQUENCIES OF AUXOTROPHIC MUTATIONS IN *S. TYPHIMURIUM* AFTER X-RADIATION AND PENICILLIN SCREENING

	1 X-Ray Dosage and Time	2 % Bacteria Surviving	3 Total No. Tests	4 Total No. Mutants	5 % Mutants	6 No. Different Mutants	7 % Different Mutants
	Strain 533						
I	Controls	100	135	7	5.1	3	2.2
II	11,400 R 4 min.	40	62	16	25.9	4	6.4
III	17,100 R 6 min.	25	86	18	20.9	4	4.7
IV	22,800 R 8 min.	14	41	11	26.8	3	7.3
V	28,500 R 10 min.	6	25	19	76.0	6	24.0
VI	34,300 R 12 min.	1.5	94	64	68.1	8	8.4
VII	45,600 R 16 min.	0.9	99	72	72.6	17	17.2
VIII	57,000 R 20 min.	1	50	34	68.0	12	24.0
	Totals	.....	459	234	.....	.....	.....
IX	II+III+IV	.....	189	45	23.8	11	5.8
X	V+VI+VII+VIII	.....	268	189	70.5	43	16.0
	Strain 519						
XI	45,600 R 16 min.	25	100	22	22.0	9	9.0

with adenine, and others (not found in this experiment) must have either guanine or thiamin in the medium.

In our published report of these data (Plough, Young, and Grimm, 1950, Table 3) we listed a number of additional strains showing alternative requirements. Davis (personal communication) retested a number of these and found them to be mixtures of single autotrophs. We have just completed an extensive recheck of all strains listed originally as alternates, and now confirm his results except for the three types of alternates listed in Table 17.2 (Plough, Miller, and Berry, 1951).

MUTATION FREQUENCY AND X-RAY DOSAGE

One of the most interesting results of this experiment is the clear relation between the frequency of auxotrophic mutants and the X-ray dosage. This is shown in Table 17.1, column 5, lines II–VIII, and I could add to the data from other experiments. The numbers of tests vary for the different radiation

dosages, and some of the values are less significant statistically, but the percentage of mutants is significantly higher at the higher dosages. This is emphasized by lines IX and X where the sums of the first three and the last four values are compared. The same conclusion is evident from inspection of column 7 in the table, where the numbers of different mutants at the successive dosages are shown. Nearly three times as many were isolated from the upper group as from the lower.

TABLE 17.2

KINDS OF AUXOTROPHIC MUTATIONS IN *S. TYPHIMURIUM*

No.	Strain 533 Single Amino Acids	No.	Strain 519 Single Amino Acids
105.....	Cysteine	8.....	Histidine
55.....	Histidine	3.....	Cysteine
15.....	Leucine	3.....	Methionine
14.....	Proline	3.....	Proline
5.....	Tyrosine	1.....	Leucine
5.....	Threonine	1.....	Tryptophane
4.....	Methionine	1.....	Phenylalanine
2.....	Valine		
1.....	Arginine		
	Nucleic Acid Fraction		
5.....	Adenine		
	Multiple Amino Acids		
1.....	Valine and isoleucine		
4.....	Unanalyzed		
	Alternative Amino Acids		*Alternative Amino Acids*
21.....	Cysteine or methionine	1.....	Cysteine or Methionine
1.....	Tyrosine or tryptophane		
1.....	Tyrosine or phenylalanine		

Line XI in the table shows the result of one radiated series made on a different initial strain, #519. Comparison of the column 5 and column 7 totals with line VII above, shows that this strain is much more resistant to radiation than is strain #533. It is clear that comparisons of the mutagenic effects of radiation dosage must always be made between samples from the same strain.

The data in Table 17.1, column 5, are graphed in Figure 17.3. Comparison of the percentages of mutants at successive dosages shows a positive correlation, though rather far from a straight line curve. As the penicillin screening method involves a 24 hour growth in complete broth, and another 24 hours in minimal medium with penicillin, it might be expected that the final percentage of mutant strains would not bear the direct relation to dosage shown in tests of mutations produced in germ cells in sexually reproducing organisms. Indeed Davis, in his account of the penicillin screening method

as used in *E. coli*, stated ". . . the method as developed so far does not appear to yield quantitative survival of mutants." Such a statement assumes that the penicillin screening may be expected to be complete, which in fact is not true. Rather penicillin acts, as do all antibiotics, in a progressive fashion according to a typical logarithmic killing curve. If two or more mutant cells

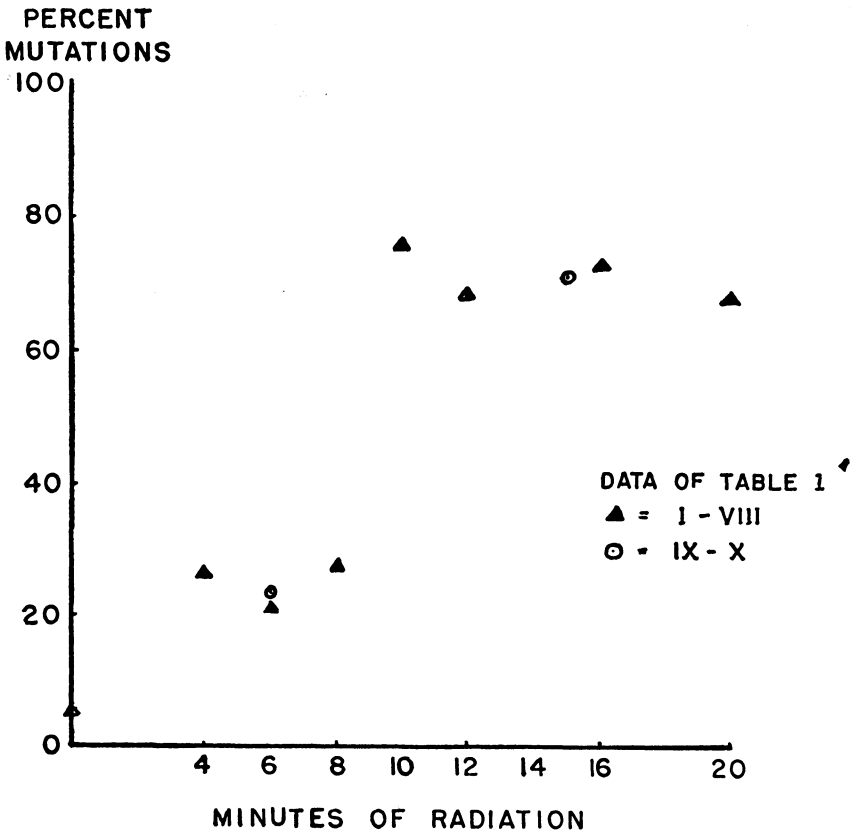

FIG. 17.3—Graph showing the relation between percentage of mutations isolated and X-ray dosage in minutes (2850 R per minute).

appear in a growing wild type population, they will increase logarithmically and form smaller less numerous clones. As the penicillin acts, the far more numerous parent clones will be logarithmically reduced in numbers, while the mutant clones exposed will have reached a level which may be maintained during the 24 hour period of penicillin action. It is clear that if a sample is taken, and plated at any point short of the complete killing off of the wild type, we may expect frequencies showing the same order as in the original population, although the mutant percentages are greatly magnified.

An actual test of artificially made mixtures of the parent strain and one cysteine requiring mutant as screened by the media is shown in Table 17.3. The data show that a mixture of 90 per cent wild and 10 per cent mutant still gives a greater number of wild survivors after penicillin screening than does a mixture having 10 per cent wild and 90 per cent mutant. For the actual experiments reported in Table 17.1 the proportion of mutants to unmutated wild type even after 24 hours of growth in complete broth is one in many thousands, rather than 10 per cent to 90 per cent. So it seems justified to consider the percentage of mutants and wild type as an index of muta-

TABLE 17.3

EFFECT OF GROWTH IN COMPLETE MEDIUM FOLLOWED BY PENICILLIN SCREENING ON ARTIFICIAL MIXTURES OF CONTROL (533) AND A CYSTEINE AUXOTROPH (533-169)

Mixture	Percentages Original Mixture		Percentages After 24 Hrs. in Broth		Percentages After Subsequent Screening	
	533	533-169	533	533-169	533	533-169
A.....	90	10	70	30	8	92
B.....	50	50	33	67	2	98
C.....	10	90	5	95	2	98

tion frequency in comparing X-ray dosages. The trend in Figure 17.3 suggests a sigmoid curve rather than a straight line as Hollaender (1948) has shown for ultraviolet induced visible mutations in fungi. Essentially the same interpretation can be drawn from a comparison of the number of different mutations found at the successive X-ray dosages. Much more extensive data are now available showing the relation between mutation frequency and both X-radiation and ultraviolet dosages and they will appear in another publication. In general they all bear out the conclusion that the frequency of auxotrophic mutations is directly correlated with radiation dosage as is true for gene mutation in other organisms.

A rather interesting result of comparison of these percentages of mutants present after penicillin screening is that the most frequent class changes from the lower to the higher dosages. Thus after 11,000 roentgens, a cysteine auxotroph is the most frequent, while after 57,000 r it is a histidine requirer. Perhaps we are dealing with a specific effect of dosage or conceivably with a differential effect of wave length, but until the complex nature of the cysteine mutants are more fully understood it is unwise to attempt too definite an interpretation.

RECOMBINATION TESTS IN SALMONELLA

Much interest has been excited among geneticists as well as bacteriologists by Lederberg's proof that mixtures of multiple mutant stocks of the K12

strain of *E. coli* give rise to new strains having the auxotrophic mutants in new combinations. These initial observations have been repeated in different combinations and amply confirmed by the observations of many other investigators. As Lederberg has suggested, these results are most reasonably interpreted as due to bacterial union like a sexual fusion of gametes, followed by an immediate reduction process involving segregation and genetic recombination, suggesting linkage in a single chromosome system. More recently Lederberg (1949) has found evidence of what appears to be a diploid strain which gives highly aberrant segregation ratios. These require assumptions of such an extremely complex and involved type of chromosome interchange that it becomes questionable whether some other explanation is not after all more probable.

In *S. typhimurium* we now have more mutant strains carrying single auxotrophic genes or multiple combinations of these than in any other bacterial species except *E. coli*. This makes it especially important to test the theory with our strains. Accordingly Miss Marie McCarthy has been mixing these in varying combinations, and then plating out in heavy suspensions on base medium supplemented so as to show up the transfer of one or more requirements from one to the other original combination.

Although more than a hundred such tests have been made and carefully checked, the results have been unequivocally negative until very recently. This work will be reported in detail in a later publication, but I will describe it briefly here. Multiple strain #519-38-94-41 requiring tryptophane, methionine, and histidine was mixed with #533-486-96-85 requiring leucine, threonine, and arginine. On plating in appropriate media it was found that in addition to the original parental combinations several colonies each gave strains requiring two new sets of requirements. Recombination No. 1 required tryptophane, leucine, and threonine. Recombination No. 2 needed all six amino acids: tryptophane, methionine, histidine, leucine, threonine, and arginine. These new stocks have been retested, and there can be no question of the fact that we have here two recombinations of the original stocks used. Other recombinations have now appeared but reciprocal classes are never found. Thus we have in Salmonella confirmation of the recombination results found by Lederberg in the K12 strain of *E. coli*. In view of the irregularity of such results both in *E. coli* and in Salmonella, it would seem wise to suggest that some alternative explanation may yet prove to be more satisfactory than recombination or chromosomal crossing-over.

BIOCHEMICAL STUDIES OF AUXOTROPHIC MUTANTS

The Neurospora studies of Beadle and his associates as well as those of Lindegren (1949) on yeast have made it evident that in studying the action of auxotrophic mutants we are many steps closer to the initial determinative activities of the genes themselves than is ordinarily true for characters in the higher plants and animals. When a series of auxotrophic genes can be shown

to block successive steps in the syntheses of particular amino acids or vitamins or more complex products, the one gene–one enzyme hypothesis offers the most satisfactory preliminary explanation, even though the presence of the particular enzyme as a gene product has not been demonstrated. Each set of auxotrophic mutants offers data on the chain of synthetic processes to some essential substance, and thus becomes a challenging biochemical problem. It is significant that many of those already studied in the fungi have also been uncovered in *E. coli*, but every organism shows individual differences. So far in Salmonella we have investigated the biochemical steps in only two such series of auxotrophs, but many others await study especially as new mutants are added.

TABLE 17.4

UTILIZATION OF SULPHUR COMPOUNDS BY VARIOUS AUXOTROPHS OF *S. TYPHIMURIUM*

Strain	Na_2SO_4	$Na_2S_2O_3$	Na_2S	Cysteine	Cysta-thionine	Methio-nine	Block in Fig. 17.4
1. Original 533.....	+	+	+	+	+	+	None
2. 533-575.....	−	+	+	+	+	−	7+2
3. 533-526.....	−	+	+	+	+	+	7
4. 533-452.....	−	−	−	+	+	−	5+2
5. 533-P249.....	−	−	−	−	+	+	4
6. 533-535.....	−	−	−	−	−	+	2

The first of these sets of interacting synthetic steps which we have studied is the cysteine-methionine auxotroph series. These mutants fall into many of the same gradations described by Lampen, Roepke, and Jones (1947) for *E. coli*, by Emerson (1950) for Neurospora, and by Teas (1950) for *B. subtilis*. We have tested all of the apparent cysteine or methionine requirers for their ability to reduce inorganic sulfur compounds as well as to utilize organic precursors of methionine. The wild type strains can reduce sulphate, sulphite, or sulfide, and can grow with no other source of S. It has been shown, however, that none of the apparent cysteine requirers can reduce sulphate, but some can reduce sulphite and some sulfide. Many, however, must have cysteine or cystathionine (kindly supplied by Dr. Cowie) and others require methionine as such.

A summary of representative mutants isolated as cysteine or methionine requirers and their abilities to grow on various compounds as the sole source of S is given in Table 17.4. This can be visualized as in Figure 17.4 in terms of a succession of steps, each catalyzed by an enzyme controlled by a gene which is inactivated by the mutation numbered in parentheses. Such a straight line series appears to run in the direction of the arrows from sulphate to protein. When a mutation occurs, as at (5), it must be assumed that growth requirements will be satisfied by any compound succeeding the break in the

synthetic chain, unless a second mutation has occurred. This does not hold for methionine which cannot be utilized in mutants #2 and 4 (Table 17.4). Such a result suggests that cysteine is enzyme controlled through a gene which is inactivated by the mutation numbered in parentheses. Cysteine is ordinarily made from methionine (as has been shown for the mammal) and so the reverse dotted arrow marked (1) is shown in the figure. It is hardly likely that a second mutation is indicated for the mutants cited as showing two blocks, but rather that certain mutations cause inhibition of more than one enzyme system. A more comprehensive scheme for the cysteine-methionine synthesis based on the Neurospora work has been given by Emerson (1950).

It is certain that more is involved in the series of reactions shown in Figure 17.4 than the furnishing of essential sulphur for cysteine and methionine.

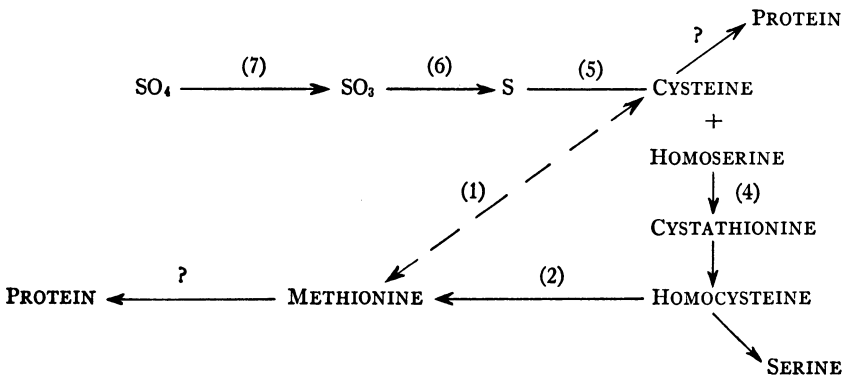

FIG. 17.4—Possible chain of reactions involving sulphur-containing compounds. (Mutant blocks indicated by numbers in parenthesis.)

Sulphate, sulphite, and sulfide, as well as cysteine itself, may act as H acceptors, cooperating with dehydrogenases involved in the respiratory or energy producing activities of the organism. That the organism reduces more sulphate than is necessary for the S required in the amino acids is indicated by the fact that Salmonella forms a readily testable excess of H_2S. We are attempting to trace the course of the sulphur by the use of the radioactive isotope S^{35}. Last summer Dr. T. P. Ting and the writer were able to show that $(NH_4)_2S^*O_4$ is taken up by the wild type 533 organisms and not at all by a cysteine requiring mutant, thus confirming our growth tests (see also Cowie, Bolton, and Sands, 1950).

We hope to continue this work using labeled sulphur in sodium sulfide or barium sulfide, which should be utilized by wild and mutants number (7), (6), (5) (Fig. 17.4). Finally it should be possible to determine by quantitative tests how much S^{35} is combined into bacterial protein and how much passed out in H_2S. Comparisons between different strains in oxygen utilization are being made with the Warburg respirometer. As already shown in

Table 17.3, some cysteine requiring strains will overgrow the parent and this may be due to differences in energy requirements.

The second set of steps in synthesis being studied concerns the adenine requirer. Here we appear to have rather more definite information than was described by Guthrie (1949) for the purine auxotrophs of *E. coli*. It has been shown that the Salmonella auxotroph utilizes adenine and hypoxanthine, but not guanine and xanthine. Of the nucleosides and nucleotides only adenosine and adenylic acid are used, and much more of the latter is required for comparable growth than of adenine. Thus it appears that in purine metabolism, Salmonella and an animal like Tetrahymena (Kidder and Dewey, 1948) show almost opposite requirements, for the bacteria do not convert adenine to guanine. Preliminary studies by Mrs. Helen Y. Miller demonstrate a sparing action for adenine utilization by the amino acid histidine. This suggests

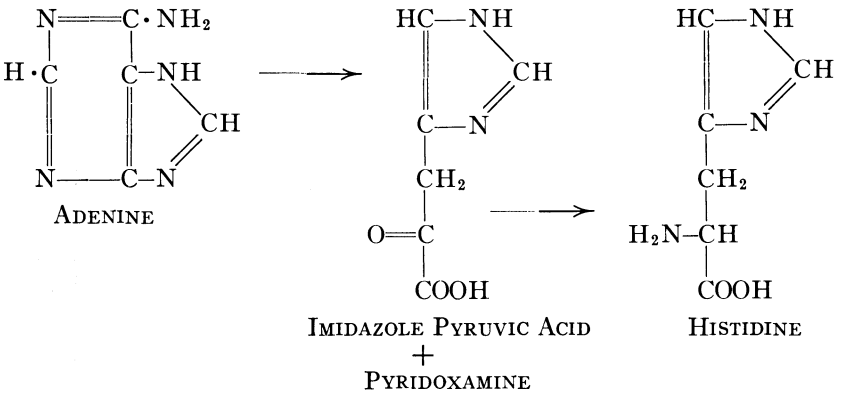

FIG. 17.5—A Possible relation of Adenine to Histidine synthesis (after Broquist and Snell).

that for this organism as with Lactobacillus (Broquist and Snell, 1949) the purine is a precursor of histidine, probably by the utilization of the imidazole ring through pyruvic acid, and the transaminating action of pyridoxamine (Figure 17.5). While these facts have been revealed by a study of the adenine mutant alone, further gene changes and their reactions with the histidine auxotrophs already available should help clarify some of the interactions of purines and amino acids in the bacterial cell.

ALTERATION OF ANTIGENIC SPECIFICITY

The auxotrophic mutations reveal a series of biochemical steps or transformations common to whole groups of organisms. Antigenic analysis, on the other hand, has revealed precise specific or strain differences which are as distinctive as the form or structural differences of complex animals and plants. This has been clearly demonstrated by the blood group analysis presented in the studies of Irwin and his colleagues. The specificity is no less

sharp in the antigenic analyses of Salmonella. The tree-like relationship which they suggest was our chief stimulus to a study of bacterial genetics in this organism.

Preliminary tests of all of the auxotrophic mutants made by Miss Dorothy Farley show that they are unchanged antigenically. Not only the specific antigens, but the agglutination titers are the same as the original strains. This has been confirmed by reciprocal absorption tests, as well as by precipitation, and inhibition of agglutination using supernatants from boiled cultures. Thus it appears that the loss of ability to synthesize a particular amino acid in no way alters the antigenic configuration. Apparently if proteins are formed at all they take on the antigenic configuration of the cytoplasm already there. The auxotrophic mutants and the antigenic patterns fall into two quite independent systems so far as present evidence goes. This seems to be true also for variations in or loss of virulence. The relation of the auxotrophic mutants to virulence for mice is being studied in detail by Gowen and his associates and will be reported separately, but so far at least it appears that there is no relation between virulence and the biochemical requirements of the strain.

It was originally and is still our hope to be able to induce antigenic variants by radiation, but so far such attempts have given negative results. We have inoculated radiated suspensions into one end of **U** tubes of semi-solid agar containing low concentrations of O serum from a rabbit immunized against the specific strain, and the organisms grow through the medium. When agar containing specific H serum is used, however, the organisms grow only at the site of inoculation. If antigenic mutants had occurred we would expect that the homologous serum would act as a screen to block off the original and let the mutants through, just as the penicillin does for the auxotrophs. The result simply means that we have not found any antigenic mutants following radiation. Perhaps we should not expect any.

Antigenic mutants have been induced in several bacteria by other methods, especially by McCarty (1946) in the pneumococcus, by Bruner and Edwards (1947) in Salmonella, and by Boivin (1947) in *E. coli*. The pneumococcus method is not applicable to Salmonella, and the Boivin method involving exposure of the organism to autolysates of rough variants of other strains gives negative results. Tests using similar culture filtrates have been unsuccessful in altering the antigenic constitution of our organism. On the other hand, Miss Farley has made use of the Edwards technique of growing an auxotrophic mutant in a semi-solid medium containing homologous O serum previously absorbed with a related organism which lacked one of the major antigens, XII (and in another case lacked V but carried an additional antigen XXVII). By this method two successful transformations of type have been secured out of several tried. Both of these transformations were performed on an auxotrophic mutant (519-P10) requiring histidine.

Preliminary tests showed that these strains were antigenically similar and gave the same agglutination titer with homologous serum as the parental wild types—(I) IV, V, XII, for the O antigens. The parentheses indicate that (I) is very weak or absent. The first case is typical. Specific serum from animals immunized by #519 was absorbed with a suspension of organisms of #527, an unnamed strain known to have O antigens IV, V only. After it was passed through semisolid agar containing the absorbed serum now carrying XII antibodies only, 519–P10 was retested and shown now to give agglutination at a very low titer (1/320 instead of 1/10,000) compared with the original. Further testing has demonstrated that this strain retains the two major O antigens (IV and V), but has lost XII. Thus it has been transformed to IV, V like strain #527. Further tests on differential media prove that the strain is unchanged as an auxotrophic mutant, and still cannot grow unless the medium contains histidine (519–10).

In the other case 519 O serum was absorbed by *S. schleissheim* (V, XII, XXVII). The mutant after growing through the absorbed serum failed to agglutinate in XII serum, and had a higher titer in XXVII than *S. schleissheim*. Thus the changed mutant has lost XII and taken on antigen XXVII. It still retains its histidine requirement.

Thus we have two independent cases of the alteration of antigenic specificity by the Edwards method of passage through specific serum. Here again the evidence indicated no relation between antigenic configuration and the biochemical requirements. We are now exposing these antigenically altered strains to further radiation with the idea of building up multiple auxotrophic stocks combining the two major systems of mutations. These can then be used for more conclusive tests of possible fusion and recombination. However, this demonstration that antigenic mutants can be induced by specific serum adds to the possibility that mutual interaction of genes or gene products between organisms in mixtures may give a more acceptable explanation of the recorded cases of recombination in bacteria, than does one based on genetic analogies with higher forms.

SUMMARY

An account has been given of the results of X-radiation of suspensions of the two strains of *Salmonella typhimurium*, and the isolation of strains with specific nutrilite requirements (auxotrophic mutants). These strains are isolated by the Davis-Lederberg method of growth for twenty-four hours in enriched broth, followed by twenty-four hours in minimal broth containing 100 units per ml. of penicillin. The method screens out the unmutated organisms according to a logarithmic survival curve, and preserves the mutant bacteria.

Successive tests show a relation between X-ray dosage and the percentage of recovered auxotrophic mutants, and also between dosage and the number of different mutants.

In all, 249 separate auxotrophic mutants, of which 20 are different, were isolated out of 459 tests. Most of these showed requirements for single amino acids, but a few required the purine base adenine, and others showed alternative, and a small number, multiple requirements.

A large number of tests involving growth of multiple mutant stocks in mixtures followed by re-isolations have been made to test for possible fusion and recombination as reported by Lederberg and others in the K 12 strain of *E. coli*. Recombination has been found but it is unlike that in sexually reproducing organisms.

Detailed studies of the different auxotrophs requiring cysteine or methionine show a step-like series beginning with loss of ability to reduce inorganic sulphate, and continuing to the loss of ability to form methionine. Many of these mutational steps are explainable as due to the inactivation of a specific enzyme, but several require a complex pattern of chemical interactions.

Similar studies of the adenine auxotroph suggest that adenine may be a source of histidine.

Tests have been made to determine if antigenic specificity can be altered by radiation, with negative results. However, an auxotrophic mutant has been antigenically altered in two different cases by the Edwards technique of passing through absorbed immune serum. In each case, one of the O antigens was removed, and in one case another O antigen was added. In both cases the biochemical requirement of histidine was retained.

It appears that the auxotrophic and antigenic series represent two quite different and unrelated sets of mutations.