zoon coccoides has not been studied. The possibility that our tissue cultures from resistant mice are resistant because of latent infection in the mice themselves seems quite remote. The factors should not, in this case, segregate in a Mendelian fashion, and latent infection of young or newborn mice has not been reported in these infections.

Summary.—A virulent strain of mouse hepatitis virus is shown to have a selective destructive effect on the macrophages cultured from the liver and other tissues of newborn mice, and no apparent effect on the fibroblasts and epithelial cells. Tissue susceptibility seems therefore to be a property of the reticulo-endothelial system. Cultures obtained from resistant strains of mice showed no destruction of macrophages, whereas susceptible strains of mice yielded macrophages which were destroyed in culture. Tests of hybrids resulting from crosses between resistant and susceptible strains indicate that susceptibility is inherited and that genetic segregation of susceptibility and resistance occurs in the F2 and backcross generations. This is apparent both in the mice themselves and in cultures obtained from the different genetic crosses.

* This investigation was conducted under the auspices of the Commission on Viral Infections of the Armed Forces Epidemiological Board, and was supported (in part) by the Office of The Surgeon General, Department of the Army.

5 Franklin, R. M., Virology, 6, 81 (1958).
10 Sabin, A. B., these PROCEEDINGS, 38, 540 (1952).

FREQUENCY OF DELETIONS AMONG SPONTANEOUS AND INDUCED-MUTATIONS IN SALMONELLA*

BY M. DEMEREC

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON†

Communicated June 21, 1960

Among mutants collected at random in microorganisms, two classes are distinguishable: those that have the capacity to revert to the wild type, and those that cannot revert. Mutants of the first class are demonstrably the result of mutation at a single site of a gene locus, whereas the nonreverting mutants are due to mutation affecting two or more adjacent sites. These so-called multisite mutants, since they show properties characteristic of the deletion mutants found in higher organisms, are assumed to originate by deletion of a small segment of the gene string.

As a rule the frequency of deletions is low as compared with the frequency of single-site mutations. In Salmonella typhimurium strain LT-2, for example, only 20 (4 per cent) deletions have been found among 495 spontaneous mutations affect-
ing 23 gene loci. An exception to this apparently general rule occurs at the cysC locus, where about 40 per cent of the spontaneously occurring mutations have been found to be deletions. Therefore cysC (cystine-requiring) mutants afford excellent material for a comparative study of frequency of single-site mutations and deletions, both spontaneous and induced. Such a study, involving mutants induced by ultraviolet radiation, 2-aminopurine, and mutator genes, will be reported in this paper.

**Material.**—Earlier biochemical and genetical studies of cysC mutants by Clowes revealed that in some the requirement could be satisfied by either sulphite or thiosulphate whereas in others it was satisfied only by thiosulphate. In the limited sample then available, the two classes formed two separate groups on a genetic map, and it was concluded that two cystine gene loci are present in that region of the chromosome, the mutants of one (cysC) being able to grow on sulphite whereas those of the other (cysD) are not. Tests of additional mutants that have since become available, however, fail to support the division into two loci on the basis of nutritional requirements, and therefore all mutations found in that region are being designated cysC mutations.

One hundred thirty-four cysC mutants were included in this analysis. All had been isolated, by the penicillin technique, from two Salmonella strains obtained from Dr. W. D. Zinder: LT-2, in which the frequency of spontaneous mutation is low, and LT-7, which has a high spontaneous mutability. Miyake has demonstrated that mutations at a mutator locus are partially responsible for the high frequency of spontaneous mutations in strain LT-7. Since LT-7 lines that carry the wild-type form of this mutator gene are still about ten times more mutable than strain LT-2, it seems probable that LT-7 may have one or more additional mutator loci. Two groups of spontaneous mutants of strain LT-7 were investigated, one carrying the mutant allele of Miyake's mutator (mut) and the other the wild-type allele (mut+). Among the mutants of strain LT-2 investigated were 46 of spontaneous origin, 21 induced by ultraviolet radiation (UV), and 40 induced by treatment with 2-aminopurine (AP).

The UV-induced mutants were obtained from bacteria treated with a dose of about 600 ergs/cm. Since about ten times as many mutants were isolated from the irradiated material as from a similar sample of untreated controls, it can be assumed that about 90 per cent of our UV mutants were induced by the radiation.

Treatment with AP was carried out as follows. Two sets of about 30 tubes each, one set containing 0.05 per cent AP in 2 ml of broth and the other (control set) containing only broth, were inoculated with about 5 x 10³ bacteria per tube. After 48 hours of incubation at 37°C, without aeration, the bacteria in each tube were washed and resuspended in saline; and 0.1 ml of each suspension was used for isolation of auxotrophic mutants by the penicillin method. Not more than one auxotroph of each kind was taken from any one tube. Since in one such experiment, made by Mr. J. Ishidsu, more than 300 different auxotrophic mutants were isolated from the treated material and only 7 from the untreated controls, it is evident that in our sample of 40 mutants probably only one was of spontaneous origin and all the others were induced by the treatment.

**Methods.**—Because the primary characteristic of deletion mutants is that they do not revert to the wild type, the first test made with every cysC mutant was to
determine whether or not it was stable. Usually instability could be detected by streak tests, that is, by observing wild-type colonies that appeared on streaks of fully grown mutant culture on minimal medium enriched with 0.01% of broth powder. Mutants not showing reversions in streak tests were further tested by plating about $2 \times 10^6$ bacteria on each of 5, 10, or more plates containing enriched minimal medium. These bacteria multiplied to form a population of about $2 \times 10^6$ cells per plate. An experiment with ten plates could detect a reversion occurring in a population of about $2 \times 10^9$ cells.

The second important property of deletion mutants is that they fail to recombine with a number of closely linked mutants. Therefore all cysC mutants which in the first series of tests appeared to be stable were investigated further by transduction experiments, in which the presumed deletion mutants served as recipients, and single-site mutants as donors. This analysis was greatly facilitated by the fact that most of the deletions identified so far are long, including many of the sites of the 91 single-site mutants tested.

By such analyses 30 deletions were recognized among 134 cysC mutations. Twenty-five of them did not recombine with a region containing 72 single-site mutations; three (cysC-36, C-47, and C-68) failed to recombine, respectively, with regions involving 34, 3, and at least 7 mutations; and one (cysC-109) covered a genetically inert region about one-quarter the length of the locus. In one mutant only (cysC-168) it has not yet been established whether the mutation is single-site or a deletion. In this case no reversion was found in tests involving about $1 \times 10^{11}$ bacteria, but cysC-168 recombines with all available single-site mutations in the region of the cysC locus where it has been mapped. It may be a highly or completely stable single-site mutation, or it may be a small deletion of adjacent sites not represented in our material. This mutant was one of the spontaneous LT-2 mutants, and in Table 1 is included in the single-site class.

A detailed analysis of the properties of cysC deletion mutants and the probable mode of their origin will be presented in another paper.

Results and Discussion.—The data presented in Table 1 show that in strain LT-2, which carries the wild type of a mutator gene, many deletions were found among the mutants of spontaneous origin and among those induced by UV treatment, but none among the mutants induced by 2-aminopurine (AP). The frequency of deletions was slightly higher in the UV-induced than in the spontaneous sample, but the numbers are too small to be significant. Only a few deletions were found among spontaneous mutants of strain LT-7. The difference between the two strains in percentage of deletions among spontaneous mutations is striking and highly significant. The data do not reveal any difference in frequency of deletions between the samples carrying mut and mut+.

The results of these experiments indicate that two basically different mechanisms are responsible for the origin of deletion mutants and single-site mutants. Both mechanisms are affected by UV radiation, as well as by the factors that bring about spontaneous mutation, but AP affects only the mechanism responsible for single-site mutants. The difference between strains LT-2 and LT-7 in frequencies of spontaneously originating deletions may be accounted for by assuming that mutator genes, present in LT-7 but absent in LT-2, were responsible for most of the spontaneously occurring mutations in the sample analyzed, and that these genes
affect only the mechanism producing single-site mutants. The evidence, presented in this paper, that all 40 mutations induced in the cysC locus by AP are single-site, whereas out of 67 spontaneous and UV-induced mutations more than 40 per cent are deletions, supports Freese's\(^5\) explanation of the origin of AP-induced mutants in phage T4. According to Freese, this base analogue can pair with thymine and may be incorporated into DNA in place of adenine. During the duplication of such DNA, mistakes in pairing are more frequent than when the usual base is present. Mutations are induced by mistakes in pairing, rather than by the replacement of DNA base by its base analogue. Accordingly, it is to be expected that AP will induce single-site mutations only.

Results of a detailed genetic analysis of the deletions in locus cysC, which will be published in another paper, suggest that they originate during duplication of the gene string, by the formation of a tight loop and the subsequent exclusion of the portion within the loop from the newly formed string. The findings indicate that a duplication about one-quarter the length of the locus is responsible for the frequent occurrence of deletion in cysC.

**Summary.**—Previous studies had shown that spontaneous mutations in the cysC locus of the Salmonella typhimurium chromosome include an unusually high proportion of deletions. Therefore this region was selected for a comparative study of frequencies of deletion mutants among spontaneous, 2-aminopurine-induced, and ultraviolet-induced mutants of strain LT-2, and spontaneous mutants of strain LT-7. The rate of spontaneous mutation in LT-7 is high because of the presence of mutator genes, one of which has been identified.

The results are given in Table 1. They show that in strain LT-2 the spontaneous

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Numbers of Single-Site Mutations and Deletions (dl) Found Among cysC Mutants of Different Origins and Genetic Constitutions</strong> (\text{(AP = 2-aminopurine; UV = ultraviolet; mut = mutator gene)})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Constitution</th>
<th>Origin</th>
<th>Mutants</th>
<th>dl</th>
<th>% dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT-2</td>
<td>mut(^+)</td>
<td>Spontaneous</td>
<td>46</td>
<td>18</td>
<td>39.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AP</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV</td>
<td>21</td>
<td>9</td>
<td>42.9</td>
</tr>
<tr>
<td>LT-7</td>
<td>mut(^+)</td>
<td>Spontaneous</td>
<td>12</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>mut</td>
<td>Spontaneous</td>
<td>15</td>
<td>1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

and UV-induced mutations included a large number of deletions (about 40 per cent), whereas none were found among 40 mutations induced by 2-aminopurine. Among spontaneously occurring mutations of strain LT-7, the frequency of deletions was low. The results are interpreted by assuming that the mechanism responsible for deletions is different from that responsible for single-site mutations, that both mechanisms are affected in cases of spontaneous and UV-induced mutation, but that 2-aminopurine and mutator genes affect only the mechanism that produces single-site mutants.

Results support Freese's\(^5\) explanation regarding the origin of 2-aminopurine-induced mutants in phage T4.

The author wishes to acknowledge the efficient assistance of Miss Carol A. Nagele in these experiments.
STUDIES ON THE HISTOCOMPATIBILITY GENES OF THE SYRIAN HAMSTER*

BY R. E. BILLINGHAM, G. H. SAWCHUCK, AND W. K. SILVERS

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, PHILADELPHIA, PENNSYLVANIA

Communicated by Sewall Wright, June 30, 1960

Although Syrian hamsters (Mesocricetus auratus) can reject orthotopic homografts of skin just as promptly and effectively as other mammals, suggestive evidence has been obtained that the number of important histocompatibility genes segregating in the various hamster stocks so far tested may be very small. For example, it has been shown that a high proportion of skin homografts transplanted between members of the same closed but random-bred stocks are usually accepted for a very long time, and that skin homografts may long be accepted even when transplanted between members of different and completely unrelated stocks. The many reported successful propagations of tumors of spontaneous or induced origin in noninbred hamsters also hint at the paucity of important histocompatibility genes in this species.

As a general rule, solid tissue homografts that establish vascular and lymphatic connections with their hosts will be exempted from a fairly prompt immunological rejection only if all the important histocompatibility genes (or transplantation antigens) possessed by them are also fully represented in the hosts. This state of affairs obtains: (a) consistently, when grafts are made within an inbred strain (isografts) or from such a strain to its F1 hybrid offspring, or (b) in a proportion of cases, when parental strain grafts are transplanted to F2 individuals. By determining this proportion ($x$) experimentally, it is possible to estimate the number of histocompatibility genes present in the one parental strain but absent in the other, since $x$ can be shown to be equal to $(n^2)$, where $n$ is the number of genes concerned. Of course, it has to be assumed that the genes segregate independently, and that each determines an antigen that is singly sufficient to provoke a level of sensitization of the host that will lead to graft destruction during the period when the animals are maintained under observation. In mice, “weak” transplantation antigens are known that may take many weeks, or even months, to procure the ultimate breakdown of homografts. Indeed, they may even fail to do so in some cases.

It must be emphasized that in the mouse, and in all other species where there are many histocompatibility genes, analyses of this sort are only possible or meaningful