some constitutions XX, X and XXX. Its most likely origin was as follows. An XX-zygote of the genotype $+car/y+$ underwent, in one of its early cleavage mitoses, a process of non-disjunction of the daughter chromosomes of the paternal X-chromosome which carried $y+$. Instead of moving to opposite poles, the two chromosomes were included in one daughter nucleus the three X-chromosomes of which carried the genotype $+car/y+/y+$, while the other daughter nucleus obtained a single X-chromosome only, with $+car$. The unchanged mitotic descendants of the XX-nuclei became the nuclei of at least the majority of the germ cells of the mosaic and probably of the cells of the whole abdomen and perhaps of parts of the thorax and head; mitotic descendants of the XXX nucleus became the nuclei of the cells of at least the lower half of the left eye and the left wing; and mitotic descendants of the X-nucleus became the nuclei of at least the right eye, the upper half of the left eye and the sex-comb-forming part of the right foreleg.

The origin of the mosaic could be explained in other ways than the one outlined here, but only by assuming a multiple sequence of abnormal processes². The single event of mitotic non-disjunction proposed above accounts for the origin of the triple mosaic in a simple way.

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¹ Morgan, T. H., and Bridges, C. E., Pub. Carnegie Inst., No. 278, 1 (1919).

² Morgan, J. V., Pub. Carnegie Inst., No. 399, 223 (1929).

Reversions induced by Base Analogues in *Salmonella typhimurium*

THE base analogues, 5-bromouracil and 2-aminopurine, employed by Freese¹ to study the *rII* region of the genetic material of bacteriophage T4 were also found to induce reversions from auxotrophy to prototrophy in *Salmonella typhimurium*. It was determined that 5-bromouracil can be incorporated into the deoxyribonucleic acid of this organism, replacing thymine specifically.

The tryptophan-D locus, known to block the last step in the biosynthesis of that amino-acid, was selected for these studies. The order of the nine mapped alleles within this locus has been established by transduction experiments and is the following²:

66

Try-D 1, 10, 35, 9, 6, 7, (55 : 69)

Each mutant strain was grown in the presence of the mutagen for about 10 divisions in minimal medium supplemented with 20 μ gm. tryptophan/ml. and 0.2 per cent glucose. The concentrations of the mutagens were: 500 μ gm. 2-aminopurine or 200 μ gm. 5-bromouracil together with 200 μ gm. aminopterin per ml. 5-Bromouracil was not effective in the absence of the folic acid inhibitor known to render the cells thymine-deficient. Following treatment the cells were washed and plated on minimal agar of two kinds: containing 0.01 per cent nutrient broth (enriched minimal) and without the enrichment.

The following results were obtained:

(a) Both base analogues increased the frequency of back mutation; from a spontaneous background of $0.5-1.0 \times 10^{-8}$, it was raised to $5-15 \times 10^{-8}$ in the presence of 5-bromouracil, and to $5-10 \times 10^{-7}$ with 2-aminopurine. The number of revertants could also be increased by plating untreated cells directly on enriched minimal agar containing 5-bromouracil and aminopterin.

(b) The increase in the number of recovered prototrophs was due to base analogue-induced mutations and not to selection of spontaneous revertants present during growth in the liquid medium. This was established in two ways: (1) by reconstruction experiments, where a tryptophan requirer was mixed with wild-type cells in known ratios; (2) by the fact that a significantly higher number of revertants was always recovered when the bacteria were plated on enriched minimal than on unsupplemented minimal agar.

(c) There was a differential response of the alleles to the mutagen. All except tryptophan D-9 and -55 reverted to prototrophy with different frequencies in the presence of the base analogues (see Balbinder³). With the exception of the mutant tryptophan D-6, all mutants which could be induced to revert by 2-aminopurine could also be induced to do so by 5-bromouracil.

Correlated with these genetic data, chemical investigations were carried out. The mutant tryptophan C-3 was cultured in enriched medium with 5-bromouracil and aminopterin. Highly polymerized deoxyribonucleic acid was isolated and estimations of the nitrogenous bases were made following the procedure described by Zamenhof⁴. On the basis of two determinations, it was found that the amount of 5-bromouracil was 30.0-33.2 mole per cent of thymine. ($5BrU/5BrU + T =$ per cent incorporation of 5-bromouracil.) Data for the incorporation of 2-aminopurine are not available.

Freese⁴ postulated that mutations of this kind are not caused merely by the replacement of a deoxyribonucleic acid base by its analogue but through mistakes in base pairing which the analogue may undergo at some constant frequency. Thus once the base analogue has been incorporated, the change in the site is dependent solely upon subsequent deoxyribonucleic acid replication. Our results tend to support this hypothesis since more revertants were recovered upon plating on enriched minimal agar, where the cells undergo a number of divisions, than on unsupplemented minimal agar.

Although the number of mutants analysed is small, the results thus far parallel those obtained by Freese. We have demonstrated that the tryptophan D locus contains base analogue-inducible and non-inducible alleles.

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² Demerec, M., Lahr, E. E., Balbinder, E., Miyake, T., Mack, C., Mackay, D., and Ishida, J., *Carnegie Inst. Wash. Year Book*, **58** (1959).

³ Zamenhof, S., *et al.*, *J. Biol. Chem.*, **219**, 165 (1956).

⁴ Freese, E., *J. Mol. Biol.*, **1**, 87 (1959).