Reversion Rate in Continuous Cultures of an Escherichia coli Auxotroph Exposed to Gamma Rays

BY R. J. MUNSON AND ANN JEFFERY

Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berkshire

(Received 23 October 1963)

SUMMARY
Continuous cultures of the tryptophan-requiring strain of Escherichia coli known as wp2 were continuously exposed to gamma radiation for periods of several generation times. The frequency of prototrophic revertants increased steadily during irradiation at a rate which was proportional to the radiation dose rate. The change of revertant frequency per unit dose (rad) was equal to the induced reversion rate per mutable unit. This mutation rate ($\rho$) was independent of population density but slightly dependent upon the presence of supplements, such as nutrient broth, to the normal minimal medium with tryptophan. There was a marked dependence of $\rho$ upon culture temperature, the values at 16°, 22° and 87° being $1.2 \times 10^{-11}$, $2.4 \times 10^{-11}$ and $8.9 \times 10^{-11}$ per mutable unit per rad. It is probable that only a fraction of radiation-induced changes in the genetic material which could give rise to phenotypic reversions are actually expressed, this fraction being dependent on the post-irradiation temperature. The proportion of the population inactivated by radiation was intentionally kept at 10% or less in order to avoid difficulties in the interpretation of results. Other possible sources of error in $\rho$ have been reviewed.

INTRODUCTION
Mutations which arise in continuous cultures of bacteria were first studied by Novick & Szilard (1950). By following the progressive accumulation of mutants induced in such cultures by mutagenic agents one can estimate the mutation rate and the delay in mutation expression (Kubitschek & Bendigkeit, 1958). The main purpose of the present experiments was to determine whether or not mutations were induced in growing bacteria with the same efficiency by gamma radiation at different dose rates and under different environmental conditions, e.g. different temperatures, population densities and growth media.

When the rate of growth of a culture is limited by restricting the supply of an essential component of the nutrient medium, as in the ‘chemostat’ of Novick & Szilard (1950) the population density and average generation time tend to constant values which are characteristic of the late log or early stationary phases of a normal batch culture. Other systems have been devised in which the optical density of a culture is automatically maintained at any desired value within the range of normal logarithmic growth, and the generation time then reaches a minimum value which is independent of the population density. Such a system has been termed a ‘turbidostat’ (Bryson, 1952).
Most previous genetic studies with continuous cultures known to the writers have been made with chemostats (e.g. Novick & Szilard, 1950, 1951; Fox, 1955; Kubitschek & Bendigkeit, 1961) and, although the theoretical behaviour of chemostatic populations has been examined very thoroughly, particularly by Moser (1958), selection pressures which exist under the prevailing conditions of extreme competition make the interpretation of experimental findings hazardous without other evidence. With turbidostatic cultures these particular selection pressures are eliminated and most of the others which remain can be investigated individually. In practice such cultures remain in dynamic equilibrium over many generation times and one can follow the small shifts from one steady state to another during periods of irradiation even with radiation levels which are so low that only a few per cent of the population are inactivated.

The usual staining procedures (Robinow, 1944) show two or more nuclei per bacterium in most strains of Escherichia coli in exponential growth, whilst X-ray survival data (Munson & Maclean, 1961) indicate that each bacterium is composed of a number of autonomous units, all of which must be inactivated to prevent colony formation. The numbers of nuclei and autonomous units are approximately equal so it may be reasonably assumed that each bacterium contains at least two complete genomes. If attention is restricted to mutations at a particular locus, a cell containing say four genomes can undergo mutations at any one of the four loci. From the radiation viewpoint each of the four loci can be provisionally regarded as a mutable unit.

Suppose that a growing population of bacteria is exposed to ionizing radiation at a dose rate $d$ for a time $\Delta$ and the frequency of mutant bacteria is measured after a period sufficiently long to allow full expression of the mutation and complete segregation of the mutable units. It can be shown (Appendix) that the increase in mutant frequency would be proportional to $d\Delta \rho$ where $\rho$ is the mutation rate per mutable unit per unit dose (rad). This increase would thus be a measure of $\rho$ if the total radiation dose $d\Delta$ were known. Alternatively one could measure the mutant frequency at intervals during an exposure lasting many generation times and deduce $\rho$ from the rate of increase of frequency which is finally reached, namely $d\rho$ (Appendix). Both methods would give the same value of $\rho$ if there were no complicating factors such as selection. One possible selective factor could arise from a difference in the bactericidal efficiency of radiation for wild-type and mutant organisms and this would become more important with increasing radiation dose per generation time. For this reason the lowest levels of radiation which gave satisfactory yields of mutants were used in the present experiments.

**METHODS**

A cylindrical lead castle with walls, floor and roof 8 cm. thick housed the bacterial cultures and two $^{137}$Cs gamma ray sources. Each source was fixed to the lower end of a sliding vertical rod and could be lowered from the 'safe' position where it was recessed within the roof of the castle to any desired position for irradiation of the cultures. When both sources were in their safe positions the castle roof could be rolled away on horizontal rails to a position clear of the culture space. In this storage position the stray radiation within and around the culture space was
Induced revertants of *E. coli* negligible (less than 5 m-rad/hr). A mechanical interlock prevented the sources being lowered unless the roof was accurately centred over the castle, so that accidental exposure of the operator was impossible.

Spaces were provided for six culture vessels $C_1$, $C_2$, ..., (Fig. 1) of the type described previously (Maclean & Munson, 1961) at 60° intervals on a horizontal circle of 10 cm. diam. centred on the vertical axis of the castle. One source $A$ was located on this axis, the other $B$ was on a parallel line 2.8 cm. away. The average dose rate within a culture was measured by an ionization chamber having a gas space of the same shape and size as the culture and which could be accurately located in the same position. The combination of the two sources gave considerable flexibility in the choice of dose rates within the range 45–1000 rad/hr.

![Fig. 1. Schematic sectional view of the lead castle with the 10c $^{137}$Cs sources $A$ and $B$, the culture vessels $C_1$, $C_2$, ..., and their corresponding reference tubes $R_1$, $R_2$, ....](image)

The system controlling the opacity of each culture, e.g. $C_1$, was based on a 'balance' of the currents in the associated photocells, e.g. $P_1$, $Q_1$, which received light transmitted by the culture and the reference tube respectively.

Light from a 80 W. lamp at the centre of the six culture array passed through each culture and thence through holes in the castle wall to blue-sensitive vacuum photo cells. In order to increase the amount of light transmitted by the culture and to minimize the amount of scattered light, lenses and suitably designed stops were included in the light channels. Possible gamma radiation damage to the photosensitive cathodes was prevented by turning the light away from the beams of stray gamma radiation with plane mirrors. Adjacent to each culture vessel, e.g. $C_1$, there was a glass tube, e.g. $R_1$, of similar size and shape filled with a solution of
ceric sulphate at a concentration dependent upon the desired optical density of the culture. The light channels for these reference tubes were similar in all respects to those for the cultures.

Each culture was maintained at an optical density close to the desired value by its own control system which functioned as follows. When a culture growing for example in C, reached the desired opacity the current through P₁ became equal to the current through the photocell Q₁ in the corresponding light channel. This equality was recognized by an electronic amplifier and a valve controlling the flow of nutrient medium which had previously been closed then opened. The optical density of the culture started to fall and when it had changed by an amount which could be detected by the amplifier the valve closed again. The number of drops passed at one opening was usually between 5 and 10 (0.33-0.66 ml.) depending upon the optical density of the culture and upon the number of drops at any one time in the supply tube to the culture. The valve opened and closed at regular intervals of a few minutes at normal growth temperatures. The two photocells associated with each culture e.g. P₁ and Q₁ were arranged in series and 50 V. was applied across them. Changes in the current through P₁ gave rise to changes in the potential of their common point and this was the signal detected by the amplifier.

Pieces of heat-absorbing glass (Chance ON 20) placed close to the central lamp prevented most of the radiant heat from reaching the cultures and this absorbed heat, together with the 80 W. generated locally, was removed by water circulating through a jacket surrounding the lamp. The water in this closed system also circulated through a parallel branch consisting of large copper coils in good thermal contact with the outside of the castle. The castle and its contents were thereby maintained at a uniform temperature. Temperature control in the range 10-40° was effected by a combination of a thermostatically controlled heater immersed in the circulating water and a refrigerating unit which maintained a constant temperature in the air space surrounding the castle.

The rate at which medium was supplied to a culture was estimated from the volume of overflow received in a given time and also by counting individual drops in the supply line using an automatic counting and timing device. A drop of medium after falling 1 cm. interrupted a light beam between a lamp and photocell (Mullard ORP 60). The resulting current pulse after amplification actuated a ratchet motor mechanically linked with a pen so that each drop caused the pen to move 0.5 mm. vertically over a recording chart. The chart was driven horizontally at a uniform speed so that the gradient of the trace indicated the flow rate. From the flow rate and the volume of the culture vessel the growth rate could be estimated (Maclean & Munson, 1961).

Organism and culture media. A sample of a tryptophan-requiring strain of Escherichia coli known as Wř2 was kindly supplied by Dr B. A. Bridges and from this a large batch of freeze-dried samples was prepared. For a single group of experiments a stock suspension was grown from one ampoule following the method of Kada, Brun & Marcovich (1961) and this was used for not more than 1 month.

Cultures were grown in a glucose salts medium (medium 'M'; Haas & Doudney, 1957) supplemented usually with 6 μg. Dl-tryptophan/ml. Other concentrations of tryptophan were sometimes used as indicated and in some experiments an amino acids pool (Kada, Doudney & Haas, 1961) or nutrient broth was added.
Continuous cultures were normally sampled by collecting a few ml. of outflow in a tube held at a constant temperature, usually 0°.

**Scoring of try+ revertants.** The number of try+ revertants/ml. was estimated in one or more of the following ways. (1) By retaining all bacteria, usually $5 \times 10^8$, from a measured volume of a sample on a ‘Millipore’ filter (0.45 μ pore size, 40 mm. diam. useful area), washing three times with 5 ml. amounts of 0.85% (w/v) NaCl, placing the filter on minimal agar (M medium in 0.75% agar) and incubating for 48 hr. at 37°. (2) By proceeding as in (1) but omitting the saline washes and either (a) incubating for 48 hr at 37° on minimal agar supplemented with 0.2 μg. tryptophan/ml., or (b) incubating for 2.5 hr at 37° (three generation times) on minimal agar supplemented with 20 μg. tryptophan/ml. and then transferring to minimal agar for 45 hr at 37°. (8) By collecting bacteria on a filter as in (1), washing them off and adding the suspension to molten agar containing M medium and tryptophan to give a final concentration in a pour plate of 0.8 μg. tryptophan/ml.

Experience showed that the number of try+ mutant colonies appearing on a Millipore filter on M agar was proportional to the total number of bacteria retained on the filter provided this did not exceed $7 \times 10^8$. On enriched plates (2(a) and 2(b)) and pour plates (8) more colonies always appeared, the excess compared with M plates being usually between 8 and 12 depending on the number of cell divisions of the try- bacteria which occurred on the filter (Glover, 1956). Allowance was made for these plate mutants in calculating mutant frequencies on enriched plates.

**Total and viable counts.** The total population density was estimated by microscopic counts in a Thoma–Hawkesley chamber and the viable count by spreading 0.1 ml. of a suitably diluted sample on nutrient agar and incubating for 24 hr at 37°.

**Statistical errors in mutant frequency.** The mutant frequency in a sample is given by the ratio of number of try+ colonies and the number of viable bacteria from which they arose. This ratio is subject to errors which are mainly statistical unless very large samples are used or mutant frequencies are high. Thus amongst a large number of replicate samples from one culture there is a Poisson distribution of the numbers of mutant colonies, so if there were an average of $M$ mutant colonies per sample the variance would be $M$. Similarly, the variance of the number of mutants per culture in a large number of parallel cultures would be equal to the average number $m$ of mutants per culture. Finally, the mutant frequency is proportional to the number $n$ of colonies (or cells) counted in arriving at the viable (or total) count of a sample, so the variance amongst replicate counts is also $n$. It follows that the purely statistical part of the standard error in mutant frequency, expressed as a fraction, is $\left(1/M + 1/m + 1/n\right)^{1/2}$.

In a typical experiment one would have numbers such as $M = 50$, $m = 100$, $n = 200$ corresponding to a fractional standard error of 0.187.

**Numbers of nuclei and lengths of organisms.** The lengths of a representative number of bacteria from each sample were routinely measured microscopically and numbers of nuclear bodies were also determined in some experiments using the methods described earlier (Munson & Maclean, 1961).
RESULTS

Continuous cultures were first grown in the absence of radiation for at least fifteen to twenty generation times under the desired constant environmental conditions. During this period each culture closely approached a steady state, the growth rate, population density and average bacterial length tending to apparently constant values. Having established this steady state gamma irradiation commenced with the lowering of the sources to positions giving the required dose rate.

Decrease of growth rate and viability during irradiation. During an irradiation lasting a few generation times the growth rate of a culture remained unchanged for the first one or two generation times then fell gradually and finally reached a constant value. The fall in growth rate was approximately proportional to the dose rate at each temperature, being 0.02/hr at 200 rad/hr at 37° and 22° and approximately half this value at 16°. After the exposure it slowly recovered to its original value. An example of these changes can be seen in the upper half of Fig. 2. The viable fraction of the population also decreased during irradiation, although, with the low dose rates used in mutation studies, the fall was less than 10% so that reliable measurements could not be made and after the first few irradiations viable counts were abandoned. Additional observations covering a much larger range of dose rates were made with strain WP2 and also with its try+ revertants and by interpolation it was possible to estimate more accurately the viabilities at the low dose rates used in the present experiments. These measurements, which form part of another experimental study to be described elsewhere, confirmed that the viabilities of try- and try+ organisms during the current experiments were indeed close to 100%.

Frequency of try+ revertants on M plates. The time course of the frequency of try+ revertants amongst the total population during a typical experiment is shown in the lowest graph of Fig. 2. Before irradiation the try+ frequency remained almost constant at approximately 10^-8 over a period of 20 hr, the spontaneous mutation rate being ~ 10^-18/bacterium/generation time. With the onset of irradiation at 425 rad/hr it rose steadily after an initial delay of less than one generation time. After irradiation the frequency remained almost constant for at least several generation times. A linear regression was fitted to the observations during irradiation, each observation being weighted inversely as the estimated variance \( \frac{M^2}{n^2} \left( \frac{1}{M} + \frac{1}{m} + \frac{1}{n} \right) \). The slope of this line corresponds to an apparent mutation rate of 1.6 x 10^-4/mutable unit/hr, which in accordance with the definition of mutation rate used above can be expressed as 3.7 x 10^-11/mutable unit/ rad. The latter should be slightly smaller than the true value of \( \rho \) since the number of try+ colonies is here compared with the sum of viable and non-viable try- organisms instead of the number of viable ones alone. The correction factor is therefore the reciprocal of the viable fraction of the whole population, which for this experiment is probably less than 1.05. An independent estimate of induced mutation rate was derived from the overall change in try+ frequency using similar weighting factors for the observations before and after irradiation. This gave \( \rho = 3.4 x 10^{-11} / \text{mutable unit/ rad.} \), no correction being necessary since the viabilities at these times were very close to 100%. 
Induced revertants of *E. coli* 197

Experiments of this type have been carried out at culture temperatures of 16, 22 and 37° using different supplements to the growth medium, a variety of exposure times and dose rates covering the whole available range. The results were subjected to a detailed statistical analysis from which values of mutation rate were found as in the example above. Those within each temperature group were compared by analyses of variances. No significant differences of mutation rate within any group were found. The combined values of mutation rate with standard errors for each temperature are given in Table 1, those based on observations during irradiation (column 3) being uncorrected for loss of viability. Evidence of a dependence of mutation rate \( \rho \) upon dose rate \( d \) was sought by fitting the observations during irradiation to a multiple regression of mutation rate on dose rate per generation time \( \tau d \) and temperature \( T \), assuming a linear relation of the form

\[
\rho = G_0 + G_1 \tau d + G_2 T,
\]

where \( G_0, G_1 \) and \( G_2 \) are constants. There was no evidence for a dependence of \( \rho \) upon \( \tau d \) although the evidence was highly significant for a dependence of \( \rho \) upon \( T \).

The residual variance about each regression was compared with the theoretical value of unity and in only two cases in eighteen was there a significant indication that the true variances differed from the estimated ones. The weighting factors used appeared to be generally appropriate and there was no evidence of large random errors other than those considered.

There was no indication that \( \rho \) was dependent upon the population density of the culture within the range \( 10^7 / \text{ml.} \) to \( 2 \cdot 5 \times 10^8 / \text{ml.} \) or upon the concentration of tryptophan between 2 \( \mu g./\text{ml.} \) and 20 \( \mu g./\text{ml.} \). The presence of a supplementary pool of amino acids (Kada, Doudney & Haas, 1961), each at 6 \( \mu g./\text{ml.} \), also had no effect. A 50% nutrient broth supplement gave a barely significant reduction of 10% at 37° and 22° whilst a single experiment at 16° showed a 30% reduction. This lack of response to supplements was not shared by the growth rate, which increased with the amino acids pool by 20% and with broth by 85%.

**Frequency of try+ revertants on enriched M plates.** After allowing for ‘plate’ mutants it was found that the frequency of induced mutants on enriched plates was always greater than that on M plates. Figure 8 shows the rise in frequency as scored on M plates (method (1) above) and M plates supplemented with tryptophan (methods 2(a) and 2(b) above) during an irradiation at 425 rad/hr at 25° in M medium supplemented with 6 \( \mu g./\text{ml.} \) of tryptophan. Both graphs appear to be of the same shape with a relatively steep initial rise indicating more than one mutable unit per bacterium (Appendix). This is consistent with observations on the average number of nuclei per bacterium.

**Distribution of population with respect to length and number of nuclear bodies.** The average length of bacteria of strain WP2 in unirradiated continuous cultures at 37 and 22°, namely 3-0 \( \mu m. \), was almost independent of population density up to \( 8 \times 10^8 / \text{ml.} \) in M medium with added tryptophan (2-20 \( \mu g./\text{ml.} \)) and was not measurably different when an amino acids pool was included. In these growth media the distribution with respect to length was closely similar to that of its parent strain *Escherichia coli* B/r when the latter was grown at 37° in minimal medium at a population density of \( 8 \times 10^8 / \text{ml.} \) (Maclean & Munson, 1961). The average number of nuclear bodies per organism in unirradiated continuous cultures at 37° was approxi-
mately 2 so that the average length per nucleus was rather larger than that previously reported for strain B/r (Munson & Maclean, 1961). At 16° the average length was 3.6 μ.

**Fig. 2.** Changes in population density (a), average length (in microns) (b), growth rate (c) and revertant frequency (try'/try'' + try'') (d), of Escherichia coli wr2 during an exposure to gamma radiation at 435 rad/hr. The culture was grown at 37° in M medium + 6 μg. DL-tryptophan/ml. + amino acids 'pool'. The fall and subsequent rise in population density occurred at the beginning and end of the irradiation and coincided with the rise and fall in average length as expected for a constant opacity of the culture. Changes in growth rate showed a delay of one to two generation times. Mutation rates were estimated from the slope of the dashed line and from the overall change of frequency.

**Fig. 3.** The frequency of induced revertants scored during gamma irradiation on M and on M + tryptophan plates. The outflows from five continuous cultures of Escherichia coli wr2 growing in M medium + 6 μg. tryptophan/ml. at 25° were pooled and samples on Millipore filters were put on M agar (method 1, ●) and on M agar enriched with 0.2 (method 2a, ○) or 20 μg. tryptophan/ml. (method 2b, △). The graphs have been drawn on the assumption that the initial rises are steeper than the final slope, i.e. that there is more than one mutable unit per bacterium. The start of irradiation and the length of one generation time are shown.

**Dependence of growth rate upon population density and tryptophan concentration.** Growth rates of cultures of *Escherichia coli* wr2 at 37° in M medium supplemented with 2, 6 or 20 μg. tryptophan/ml. were measured at different population densities between 10^7 and 10^9/ml. Growth rates were the same at all three tryptophan concentrations and all population densities up to 2.5 × 10^8/ml. but, as the population density was further increased, the growth rate with 2 μg. tryptophan/ml. diminished rapidly and became almost zero at 2.7 × 10^9/ml. This sudden fall could only be interpreted in terms of tryptophan exhaustion at 2.7 ×
Induced revertants of *E. coli*

10⁹/ml. For the higher concentrations of tryptophan curves very similar to those found previously for *E. coli* b/r (Maclean & Munson, 1961) were obtained, the growth rate falling gradually at densities greater than 3 × 10⁹/ml. One may conclude from these observations that there was no limitation of growth due to deficiency of tryptophan or other constituents of the growth medium at the population densities usually employed in the present experiments.

DISCUSSION

The broad conclusion from the present experiments is that environmental factors other than temperature have an insignificant or marginal effect on the induced mutation rate for the reversion try⁻ to try⁺ in *Escherichia coli* strain WR2. Since the temperature factor is important in any theory of the nature of gene mutations, possible outstanding sources of error in the data of Table 1 may be considered. The first of these is the selective advantage or disadvantage of try⁺ organisms particularly those induced in the early stages of an irradiation. In most experiments the number of revertants induced during say the first quarter of the irradiation period was between 10 and 100, and, if by chance an appreciable fraction of these had a marked selective advantage or disadvantage, the gradient of the graph of try⁺ frequency, as in Fig. 2, would have altered with time. No statistical evidence of curvature of these graphs was found, the dispersal of the observed mutant frequencies about the regression lines being within the expected limits in 16 out of 18 cases.

After irradiation, significant increases or decreases in try⁺ frequency sometimes took place over a period of 30 or 40 generation times but these changes corresponded to weak selection pressures which would have been too small to produce significant changes in apparent mutation rate during the irradiation period.

Although viable counts have shown that the lethal effect of continuous gamma irradiation was small, it is satisfactory to find that the discrepancies between estimates of mutation rate by the two methods, only one of which was affected by loss of viability, are also small. One may therefore conclude that neither selection nor the lethal action of gamma radiation influenced the observed mutation rates substantially.

Lastly, when cultures of this strain are held in a state of arrested growth and exposed to rather large flash doses of ultraviolet light or X-rays, the expression of the mutation try⁻ to try⁺ is dependent upon a number of environmental factors during the first generation time of renewed growth (Witkin, 1956; Doudney & Haas, 1959; Kada, Doudney & Haas, 1961; Kada, Brun & Marcovich, 1961). The present experiments have shown that two such factors, namely, the presence of tryptophan and the temperature, are also effective with actively growing cultures exposed continuously to low-level gamma irradiation. In Fig. 3 the initial rise of try⁺ frequency as estimated on enriched plates precedes the rise on minimal plates by about half a generation time. This result is to be expected if the genetic function of newly induced mutants is expressed during residual growth on enriched agar after plating and by contrast colonies on minimal plates could arise only from mutants in which genetic function was fully expressed before plating. The time interval separating the two parallel graphs would then represent the time required for expression of newly induced mutants in the growth medium apart from possible complications, e.g. the continuance of processes leading to expression despite lack
of exogenous tryptophan. This phenotypic delay will have no effect however upon the gradient of the graphs provided the delay remains unchanged.

As a result of changes in the routine of sample collection it became evident that there was a progressive fall in the score of try+ colonies from samples taken during an irradiation and held at 0° for some hours. Errors in try+ frequency arising from this were minimized by collecting for a constant time and keeping this as short as possible. Samples taken before irradiation and a few generation times after irradiation did not show the effect. One may therefore assume that only newly induced mutants were susceptible and if so no error was incurred in the estimates of mutation rates.

Thus it appears that the lethal effect of gamma radiation is the only likely source of systematic errors and this only affects the results for mutation rate derived from the slopes of the graphs. By allowing an arbitrary but not unrealistic average figure of 5% for this error one obtains the 'best' values for ρ given in the fifth column of Table 1.

Table 1. Generation times and induced mutation rates from try− to try+ for cultures grown in M medium + tryptophan at different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Generation time τ (hr)</th>
<th>Mutation rate/mutable unit/μrad × 10⁻¹¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>From slope 4-06±0-16 3-89±0-18 2-8</td>
</tr>
<tr>
<td>37°</td>
<td>0-72</td>
<td>3-60±0-12 4-06±0-16 3-89±0-18 2-8</td>
</tr>
<tr>
<td>22°</td>
<td>2-0</td>
<td>2-28±0-12 2-53±0-38 2-42±0-20 4-8</td>
</tr>
<tr>
<td>16°</td>
<td>5-0</td>
<td>1-13±0-12 1-04±0-16 1-13±0-17 5-5</td>
</tr>
</tbody>
</table>

The uncorrected estimates of gamma ray-induced mutation rates in the third column have been combined (after allowing for a 5% error, see Discussion) with those of the fourth column to give the 'best' values in the fifth column. The mean values and their standard errors were derived from the following numbers of experiments: 11 at 37°, 7 at 22° and 7 at 16°.

Few investigations have been reported on the dependence of mutation rate upon temperature for bacteria. Anderson & Billen (1955) using large flash doses of X-rays found that the absolute numbers of induced revertants amongst four auxotrophic strains of Escherichia coli were greatest between 18 and 24°. In retrospect their results seem of dubious significance mainly because revertant colonies were scored on minimal agar plates which did not permit protein synthesis and thus probably inhibited expression. Spontaneous reversion rates of Escherichia coli 15 his− were measured with some precision by Ryan & Kiritani (1959), who concluded that the product of mutation rate and generation time was constant between 15 and 37°, in accordance with prediction from the copy-error hypothesis. This constancy was not found with induced reversions of E. coli WP2 try−, as may be seen from the figures in the last column of Table 1.

Although the 'loss' of newly induced try+ revertants has only been observed in samples kept at 0° for some hours, it is likely that a small proportion will also fail to express themselves at higher temperatures. Thus the sharp fall in mutation rate between 22 and 16° may be related to failure of expression during the average period of τ/2 between the initial damaging event by radiation and the collection of the bacterium in the sample of outflow. Unless a similar explanation is invoked for the whole temperature range up to 37°, one would be forced to conclude either
that the initial damage, presumably to DNA, is also subject to repair or restitution at a rate which is temperature dependent or that this initial damage is less severe at lower temperatures.

APPENDIX

The induction of mutants in bacterial populations exposed to ionizing radiation for very short times (flash doses) and also continuously over many generation times will now be examined.

For simplicity it will be assumed that all bacteria of a synchronously dividing population are identical and that each undergoes binary fission to give two equal daughters at regular intervals T. If each bacterium has p mutable units at the time of a flash dose D (i.e. at zero time) and the mutation rate per mutable unit per rad (unit dose) is ρ when averaged over one generation time, the probable number of gene mutations in the viable population of N cells is DρpN. Since the mutations occur randomly the probability of two occurring in the same cell is Dρ²p², which in practice is negligibly small.

When bacteria are plated before cell divisions occur and each mutated gene gives rise to a mutant colony the apparent frequency of mutant bacteria amongst the population, namely (number of mutant colonies)/(number of viable bacteria plated) is Dρp. If, however, the population continues to grow and divide for a time T before plating it would then number 2N whilst the number of mutant cells would still be DρpN, assuming that the processes leading to mutation are irreversible and p ≥ 2. The mutant frequency at time T would then be Dρp/2. If p = 2 where r is an integer then segregation of mutant genes would only be complete at time rT, the mutant frequencies at times 2T, 3T, ..., rT being Dρp/2¹, Dρp/2², ..., Dρp/2ʳ. At times later than rT, the mutant frequency would be unchanged at Dρp/2ʳ, i.e. at frequency Dρ, since p = 2. This is identical with the frequency of mutated genes immediately after their irradiation, namely Dρp/pN = Dρ.

Let us suppose that a continuous culture consists of bacteria each of which has either p or 2p mutable units per cell depending upon the relative phases of the replication and division cycles. We assume that all bacteria both mutant and non-mutant divide and replicate their genes at regular intervals T, but since the population is asynchronous with a completely random distribution of division times the numbers of bacteria with p and with 2p mutable units per cell will always be constant apart from statistical fluctuations. Let N₁ and N₂ be the numbers of viable cells with p( = 2) and 2p( = 2⁺) units respectively and let ρ, the mutation rate per mutable unit per rad, be assumed to be independent of radiation dose rate. If irradiation at dose rate d starts at zero time then at time t (where t > r + 1r) the numbers of mutants arising from the two classes of bacteria, namely m₁ and m₂, will be increasing at the rates

\[
\frac{dm_1}{dt} = N_1ρpd - \frac{1}{2}N_1ρpd - \frac{1}{2^2}N_1ρpd \ldots - \frac{1}{2^r}N_1ρpd,
\]

and

\[
\frac{dm_2}{dt} = 2N_2ρpd - N_2ρpd - \frac{1}{2}N_2ρpd \ldots - \frac{1}{2^r}N_2ρpd,
\]

where in each equation the first term represents the rate of induction of new mutants at time t, the second term the rate of loss in the outflow of mutants which
were induced at time $t - \tau$, the third term the rate of loss of mutants induced at time $t - 2\tau$, ..., and the last term the rate of loss of those induced at time $t - \tau r$ in the case of equation (2) and at time $t - r + 1\tau$ in the case of equation (8). Mutants induced at times before $t - r + 1\tau$ will have completely segregated at time $t$ and will not contribute additional mutants to the steady population since they divide at the same rate as non-mutants.

It can be readily shown that the right-hand side of equation (2) is $N_1 p \rho d / 2\tau$ and of equation (3) is $N_2 p \rho d / 2\tau$, but since $p = 2\tau$ the rate of production of mutants from both classes of cell is

$$\frac{dm_1}{dt} + \frac{dm_2}{dt} = (N_1 + N_2) \rho d = N \rho d. \tag{4}$$

Thus at times greater than $r + 1\tau$ the mutant frequency increases at a rate equal to the mutation rate per mutable unit, irrespective of the generation time or of the interval between cell division and replication of the gene concerned.

The above argument with slight modifications would apply if $r$ were non-integral and unequal daughters arose at division and so the conclusion is valid under most conditions encountered in experimental continuous cultures for both induced and spontaneous mutations.

The mutation rate per mutable unit is also simply related to the change in mutant frequency resulting from the irradiation. If the time of irradiation were $\Delta$ and the total dose to the culture $\Delta d$, the mutant frequency measured at times later than $\Delta + r + 1\tau$ would be greater than the initial frequency by $\Delta \rho d$ (as for a flash dose $\Delta d$), since mutational damage is assumed to be irreversible and independent of the dose rate.

At the beginning of an irradiation the mutant frequency increases at a rate greater than $\rho d$ by a factor equal to the average number of mutable units per bacterium since the segregation of mutable units during the first small fraction of a generation time is negligible. The rate of increase of mutant frequency thereafter falls steadily until it reaches the value $\rho d$.

An important factor neglected in the above analysis is selection. If the mass growth rate (Powell, 1956) of the whole culture were $\mu$ and the selective advantage of the mutants were equivalent to a difference, real or apparent, of $\mu_m - \mu$ in growth rate, then the rate of increase in the number of mutants $m$ at times greater than $r + 1\tau$ would be given by

$$\frac{dm}{dt} = N \rho d + (\mu_m - \mu) m, \tag{5}$$

which has the solution

$$m = N \rho d (e^{\mu_m - \mu} - A) / (\mu_m - \mu), \tag{6}$$

where $A$ is a numerical constant. The number of mutants would therefore change with time in a perceptibly non-linear manner unless $\mu_m - \mu$ were small.

Advice on a suitable design for the amplifiers of the control equipment was kindly given by Mr F. S. Williamson. Much of the apparatus was designed and made by Mr R. J. C. Hudson. To them both we wish to express our gratitude. We also acknowledge the help of Mr D. G. Papworth, who was responsible for much of the statistical analysis.
Induced revertants of E. coli

REFERENCES


