Mutagenicity of radiations at low doses

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As a result of large scale application of atomic energy, there is public concern about the effects of ionizing radiations. The interest is mainly centered on low doses, of the order of 1 mGy* or less.

At intermediate – high radiation doses, experimentally induced effects with a genetic mechanism (mutation, cancer) often exhibit a linear dose response that may be extrapolated through the spontaneous frequency at dose zero. It has, therefore, become a practice in risk estimates to assume the dose-response curve to be linear all the way down to very low doses:

\[ R = C + \alpha D \]  

[\( R = \text{response}; C = \text{spontaneous frequency}; \alpha = \text{slope}; D = \text{dose} \)]

It has also become a practice, on the basis of studies of specific locus mutations in mice (Russell et al. 1959), to consider the risk to be approximately three times lower at dose rates appreciably lower than those usually obtained with standard equipment, of the order of 1 Gy min\(^{-1}\) (cf. UNSCEAR 1977).

Both these assumptions rest on weak grounds. A number of experiments indicate deviations from linearity at low doses or low dose rates. "Humped" dose-response curves, i.e. with a mutagenic effectiveness higher than expected from linear extrapolation, have been observed, e.g. in Drosophila (Offedal 1964a, b, 1968), in barley and maize after irradiation of meiotic stages (Ehrenberg and Eriksson 1966; cf. also de Netancourt et al. 1977) and in yeast (Eklund 1977), and it has been suggested that the mutagenic effectiveness of low-LET radiation is again higher at dose rates below some critical value, around 10\(^{-1}\) Gy min\(^{-1}\) (Lyon et al. 1972). In other materials, no deviation from linearity has been observed (e.g., for somatic mutation in Tradescantia stamen hairs at doses down to 2.5 mGy of X-rays or 0.1 mGy of neutrons (Sparrow et al. 1972), and in still other experiments even a beneficial effect of low-dose radiation has been observed (see refs. in Ehrenberg 1978). A number of indications are further at hand that the carcinogenic effectiveness of radiations in man might increase with decreasing dose or dose rate (Baum 1973; Brown 1977; Ehrenberg 1978). It is evident that the evaluation of the genetic risks from environmental chemicals encounters the same problems.

Especially in mammalian systems, experiments

* 1 Gy (gray) = 1 J kg\(^{-1}\) = 100 rad; 1 milligray of low LET radiation corresponds to an average annual dose of background radiation received by human beings.
at those low doses or dose rates that are of practical interest become prohibitively expensive, if not impossible. For improved risk estimations at these low doses/dose rates, an understanding of the mechanisms of deviations from linearity is required. Research aiming at such clarification has to be carried out in suitable microbial systems and the validity of the obtained results for estimation of risk to man must be investigated.

In studies with such aims, it is important to realize the quantization of dose, as a basis of a definition of "low dose" and "low dose rate". If, during X-irradiation, a mammalian cell or cell nucleus is hit by a photo- or Compton electron, it receives a dose of the order of $10^{-3}$ Gy (0.1 rad) or $10^{-2}$ Gy (1 rad), respectively. If one hit in any of these structures changes the sensitivity to further hits, doses below the given values may be considered "low". If the sensitivity change is reversible, say, within 24 h, then dose rates below about $4 \times 10^{-5}$ or $4 \times 10^{-4}$ Gy h$^{-1}$, respectively, would have to be considered low. A deviating effectiveness of low doses and low dose rates might evidently have the same mechanism.

The present paper describes some preliminary studies of the dose response of induced mutation in *E. coli*. The studies aim exploratorily at an evaluation of the suitability of this system for a clarification of mechanisms of deviations from linearity at low doses/dose rates. Already 20 years ago, Demerec and Sams (1959) obtained strong indication that reversion to prototrophy at three loci of *E. coli* was more effectively induced by lower doses of X-rays. Due to the smaller size of the bacterial "nucleus", one hit is associated with the absorption of a dose in the range of 0.2-1 Gy. In the present studies the effectiveness of 0.75 Gy was compared with that of higher doses.

Material and methods

**Bacteria.** – *Escherichia coli* Sd-4, a streptomycin dependent strain, obtained from Professor G. Bertani, Stockholm, was employed; mutation to streptomycin non-dependence was studied. A brief review of the streptomycin dependence system is presented as an Appendix.

**Radiation.** – X-irradiation was carried out at a Siemens X-ray apparatus operated at 180 kV and 15 mA; the dose rate being 1.05 Gy$^* \text{ min}^{-1}$. γ-irradiation was performed in a $^{60}$Co gamma-cell at 40 Gy$^* \text{ min}^{-1}$. Doses in the range of 0.7–100 Gy were given.

**Media.** – Culturing broth contained per litre the following components: Bacto trypton, 10 g; Bacto yeast extract, 5 g; NaCl, 10 g; glucose, 1 g; streptomycin, 50 mg.

For plating, 15 g Bacto agar was added to the above broth which contained streptomycin when the purpose was determination of survival; streptomycin was omitted for the mutation count. Buffer: 0.1 M phosphate buffer, pH 7.

**Culturing and treatment.** – The overnight culture was washed twice with buffer and suspended in the same at around $10^8$ cells ml$^{-1}$ for irradiation of cells in stationary phase. After irradiation the cells were plated directly for mutation and survival count. In studies of bacteria in log phase, the overnight culture was diluted 1:50 in fresh broth and cultured to log phase.

**Statistical evaluation of the data.** – Since we are dealing with small effects, any evaluation of the data would have to include errors of mutation frequency and survival (or the number of bacteria plated) of control and treated groups. Details of the method for the statistical treatment will be given elsewhere. The following equations for the calculation of the standard deviation, $\sigma_{dM}$, of the difference, $d_M$, between irradiated and control samples was used:

$$
\sigma_{dM} = \sqrt{(M_i)^2 \left( \frac{1}{\Sigma x_i} + \frac{1}{\Sigma y_i} \right)} + (M_c)^2 \left( \frac{1}{\Sigma x_c} + \frac{1}{\Sigma y_c} \right)
$$

$M_i$, $M_c$ = Mutation frequency of irradiated and control samples, respectively

$\Sigma x_i$, $\Sigma x_c$ = Sum of surviving bacteria in irradiated and control samples, respectively

$\Sigma y_i$, $\Sigma y_c$ = Total number of mutants counted in irradiated and control samples, respectively

In each experiment, Poisson distribution of $x$ and $y$ was certified by the demonstration that $x/s^2$ and $y/s^2$ were not significantly different from 1.

The variances were weighted and pooled for all the experiments, and then coefficients of variation were calculated for the doses in the 0.75 Gy range and those in the 3 to 12 Gy range. The confidence limits of the quotient between mutations per Gy at 0.75 Gy and mutations per Gy at 3 to 12 Gy were calculated for the pooled data.
Results and discussion

Fig. 1 presents the pooled data from six tests with X- or γ-rays. Though the points bear large 95% confidence intervals, the mutagenic effectiveness (mutations per Gy per $10^8$ bacteria) is indicated to be highest at 0.75 Gy and tends to decrease continuously to about 5 Gy. At doses above 50 Gy it rises again. The quotient between the mutagenic effectiveness at 0.75 Gy and that in the intermediate dose region – 3 to 12 Gy – was calculated to be 1.692 with the 95% confidence limits 1.265 and 2.119. In agreement herewith, the doubling dose for mutation to streptomycin nondependence on the basis of the effectiveness at 0.75 Gy would be about 3 Gy, whereas on the basis of the effectiveness at 12 Gy it would be about 5 Gy.

It seems reasonable to assume that the increasing effectiveness at high doses (see Fig. 1) could be a result of the saturation of the repair systems and partly due to other effects, but a high effectiveness at low doses is more difficult to explain. A general stimulatory effect of radiations has been often observed in plant materials, under conditions so far not well defined (Saleh et al. 1978). Perhaps a higher mutagenicity at low doses should also be seen in the same context. Several explanations for such an observation with respect to other
systems have been forwarded: (1) A mathematical model based on the correlation between sensitivity to killing and mutational response in heterogeneous populations has been applied by ØSTEDAL (1968, 1974) and EKLUND (1977) to explain the humped curves in their materials (Drosophila and yeast). The model as originally forwarded by ØSTEDAL (1968) assumes that the population consists of a sensitive and a resistant fraction; a low dose evokes response from both the sensitive and the resistant populations, whereas at higher doses the sensitive fraction is eliminated, with a decrease of the average mutational response per survivor in consequence. Though this model suits their data, it is difficult to apply to our system due to the lack of resolution of the population into sensitivity fractions in the dose region under consideration. It seems plausible that part at least of a supralinear effectiveness of low doses or low dose rates in cancer initiation, could have a mechanism of this kind (cf. BAUM 1973; BROWN 1977; EHRENBERG 1978, and quoted papers). (2) It has been suggested (EHRENBERG and ERIKSSON 1966) that the supralinear hump observed in higher organisms could be a consequence of a radiation-induced delay of development, different for cell fractions of different radiosensitivity. The hump around 0.1 Gy, reported by JENSEN and RAMEL (1976) to characterize the dose response of micronuclei in polychromatic erythrocytes could, in later studies (JENSEN and RAMEL 1978), be given an explanation of this kind, the effect disappearing if “cumulative” fixation was done at different times. This explanation cannot be valid for the similar hump of the dose response curve of waxy mutations in pollen grains of barley. In these studies all pollen grains reaching maturity were collected (de NETTANCOURT et al. 1977); besides, the effect was pronounced also at low dose rates of chronic irradiation (EHRENBERG and ERIKSSON 1966).

In the bacterial system of the present study, it is most probable that every mutated bacterium, even if its development is delayed, would have equal chance of developing a colony in the mutation test. (3) With reference to biochemical changes observed at doses below 10^-2 Gy, EHRENBERG and ERIKSSON (1966) suggested, as an additional possibility, that such changes could include a changed radiation sensitivity. Evidence is now accumulating that repair enzymes are inducible; this concerns error-free repair enzymes induced by alkylating and/or arylating agents (MACNAUGHTON and WINDER 1977; SAMSON and CAIRNS 1977) as well as enzymes involved in error-prone repair by UV, X-rays, alkylating agents and other agents that cause a temporary block of normal replication (DEFAIS et al. 1971; RADMAN 1973; see also WITKIN 1967). A supralinear hump of dose-response curves for mutation, as found in the present study would be compatible with inducibility of enzymes involved in error-free excision repair. Similar conclusions were recently drawn by ALTADAN and TUSCHL (1978) with regard to genetic effects in man. In the case of inducibility of error-prone repair, increased survival is gained at the cost of increased mutation frequency at doses above the one required for enzyme induction.

The E. coli system of the present study is suitable for an analysis of the dose-response curve for mutation at low doses, and of the mechanism of a positive deviation from linearity at these doses.

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JENSEN, D. and RAMEL, C. 1978. Factors affecting the induction of micronuclei at low doses of X-rays, MMS and di-
The dependence on streptomycin is known in several species of bacteria, but a detailed genetic information on this phenotype is available only for *E. coli*.

Normally the *E. coli* bacteria are sensitive to streptomycin; therefore, the mutations to resistance and dependence are forward changes. The mutation from dependence to non-dependence, an 'end-point change', is however, not strictly a reverse change as would appear from the following. Studies on streptomycin dependence in *E. coli* were initially carried out by Demerec's group (DEMEREC et al. 1949, 1950; DEMEREc 1950, 1951) and continued by Bertani (1951) who investigated the pattern of response of some of the dependent strains, particularly *E. coli* Sd-4 under a variety of conditions. These studies revealed that each strain had its own spontaneous reversion rate; that the expression of mutation to non-dependence occurred by about the sixth residual division in all the strains; and that recombination could not occur in crosses between the different strains. It was demonstrated in further studies by Demerec and coworkers (DEMEREC et al. 1951; DEMEREc and HANSON 1951) and by Szymbalski (1958) that the streptomycin dependent system could be employed for the detection of genotoxic effects of a variety of agents – possibly a consequence of the ability of the dependent strain to undergo reversion to nondependence by more than one pathway.

The gene responsible for streptomycin dependence 'str' codes for the ribosomal protein, 30S-15. The streptomycin region in the genetic map includes the majority of the genes coding for ribosomal proteins of both the 30S and 50S fractions (Takata 1972). Among these genes there are many, for example the 'rpxD', with suppressive effects on 'str' (Birge and Kurland 1969, 1970; Kreider and Brownstein 1971, 1972). Mutation in any of these linked suppressors (DEMEREC et al. 1956; Hashimoto 1960; Brownstein and Lewandowski 1967; Apirion et al. 1969) would result in a suppression of the dependent phenotype. As to the types of change, these suppressor genes seem to permit considerable latitude, e.g., insertions, deletions and transitions could occur resulting in protein chains of various lengths and proteins with amino-acid alterations (Hasenbank et al. 1973). These changes in proteins would affect the ribosomal assembly, thereby eliminating the need for streptomycin during the translational activity (Olsson et al. 1974).

It is of interest that mutation through suppression is indicated to be the main pathway for the change from dependence to nondependence.

**Appendix**

**Streptomycin dependence**

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The dependence on streptomycin is known in several species of bacteria, but a detailed genetic dependences on streptomycin is known in several species of bacteria, but a detailed genetic...