EFFECTS ON GENETIC RECOMBINATION OF ESCHERICHIA COLI K-12 PRODUCED BY X-RAY AND ALPHA-PARTICLE IRRADIATION OF THE FEMALE[1]

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THE appearance of recombinant cell types in *Escherichia coli* K-12 has been shown to depend on a sequence of consecutive steps: (1) an effective contact between two cells of opposite mating types; (2) a transfer of a portion of the genetic material from the donor cell to the recipient cell; (3) an integration of the genetic information from both parental strains into a recombinant cell with the subsequent production of a clone. (See CLARK and ADELBERG 1962 and JACOB and WOLLMAN 1961, for reviews.) High frequency recombination strains (Hfr) are able to transfer to the recipient cells with high frequency genetic determinants in a sequential order which depends on the particular strain. The probability of transfer of a given character decreases with its distance from the portion of the chromosome first injected (the origin). Two alternative, but not mutually exclusive, mechanisms have been considered for the integration processes which are involved in the production of recombinants: (1) an interchange of DNA fragments between the two parental chromosomes (Breakage-and-Reunion); and (2) a *de novo* synthesis of a recombinant chromosome by copying alternatively the base sequences from the two parental chromosomes (Copy-Choice).

Perturbations applied to the genetic material of the parental cells might affect selectively the various steps in the recombinational process. Earlier studies (MARCOVICH 1961; WOOD and MARCOVICH, unpublished) have shown that X-ray and alpha-particle irradiation of the donor strain results in a decreased probability of effective transfer of the male markers to the zygote, the radiosensitivity for this process being directly proportional to the distance between the origin of the chromosome and the selected marker. An analysis of the unselected markers reveals that no measurable radiation damage at moderate doses is carried on the material transferred to the zygote, the transfer process *per se* effectively eliminating radiation induced lesions by causing them to be expressed operationally as breaks that prevent their transfer. Hence X-ray or alpha-particle irradiation of the donor cells gives no information on those steps in the recombination process subsequent to transfer of genetic information to the female.

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However, if the recipient cells are irradiated before mating, the transfer process *per se* is not found to be measurably affected, and any change in the relative frequencies of appearance of the various recombinant types is therefore due to modifications brought about by radiation in those steps subsequent to transfer. In the experiments to be reported, X- or alpha-irradiation of the recipient cells before mating causes drastic changes in the recombinational frequencies for the various markers utilized in the cross as if there were a preferential utilization of the male genetic information.

MATERIALS AND METHODS

Strains: Escherichia coli K-12. *Recipient:* F⁻ PA-309 (*thr, leu, try, his, arg, thi, lac, gal, mtl, xyl, mal, T1ʳ, strʳ*), where the abbreviations symbolize threonine, leucine, tryptophan, histidine, arginine, thiamine (vitamin B_1), lactose, galactose, mannitol, xylose, maltose, coliphage T1, and streptomycin, respectively. The gene symbols as written above indicate that the strain shows dependence for the various amino acids and thiamine or cannot use a particular sugar in lieu of glucose as an energy source; *s* and *r* indicate sensitivity or resistance of the strain to an agent. *Donor:* HfrH (*thi, strˢ*). The markers used in this study were: *thr*+(8), *leu*+(8.5), *gal*+(24), *try*+(33), and *his*+(59), where the numbers in parentheses refer to the minimum time in minutes required for the transfer of the marker from the male (HfrH) to the zygote. Streptomycin sensitivity is transferred with low frequency to the zygote under the conditions used here.

Media: M medium: the minimal synthetic medium previously described (MARCOVICH 1961) plus 0.4 percent glucose and 4 μg/ml thiamine. Tryptone Broth: nutrient broth (Difco) 0.3 percent, bacto tryptone (Difco) 0.5 percent, NaCl 0.5 percent. For solid media, agar was added at a concentration of 1.5 percent. Selective solid media: M medium plus agar fortified with streptomycin 60 μg/ml, leucine 30 μg/ml, threonine 30 μg/ml, tryptophan 8 μg/ml, histidine 8 μg/ml, and arginine 30 μg/ml. For characterization of the markers utilized here, one of the amino acids threonine, leucine, tryptophan, or histidine was omitted or galactose was substituted for glucose at equal concentration.

Mating and assay: Male and female cells were grown in broth to a concentration of 2 to 3 $\times 10^8$ cells/ml (exponential phase). Aliquots of the female cells were pulled down as single layers on 25 mm millipore filters (type HA), irradiated, and subsequently removed from the filter by agitation in 3 ml of M medium. To facilitate the removal of the cells from the filters, base layers of *E. coli* B (*strˢ*) cells were pulled down on the filter before the female cells. Recovery efficiencies with this technique were 80 ± 10 percent. Hfr cells were washed on millipore filters and resuspended in M medium.

Aliquots of male and female cells totaling 2 ml were mixed together for mating and gently agitated at 37°C for 90 minutes. Since the irradiated female cells are damaged at random, males might conjugate preferentially with the least damaged ones. This bias would be greatest at low mating ratios (the ratio of Hfr to F⁻ cells in the mating mixture). Variations between 1/20 and 20/1 in the mating ratio do not affect any of the results reported here; a mating ratio of 1/20 was used generally in these experiments. All calculations were made with respect to the input number of the minority parent in the mating mixture.

After proper dilution, aliquots were plated on the various assay media and the numbers of colony-forming units were counted after incubation at 37°C for 48 hours. The presence of the unselected markers among recombinants (genetic constitution) was determined by inoculation of colonies from the selective plates onto a master grid plate. After growth, these colonies were replicated on various test plates.

Irradiation: The alpha particle source (30 millicuries of Po^{210}, deposited on a nickel disc 1 cm in diameter and located 20 mm from the millipore filters) gave a corrected dose rate of approximately 20 kilorads/min as determined by calculation. The X-ray source (a Holweck tube with molybdenum target, 0.05 mm aluminum filtration) gave a dose rate of 43 kilorads/min at an

anode-to-filter distance of 56 mm when operated at 37.5 kv and 40 ma as determined by ferrous sulfate dosimetry.

Additional information on the experimental procedure is given in the legends for the figures.

RESULTS

Effect of irradiation on recombinational frequencies of selected markers: To best illustrate two different points, data for X-ray and alpha-particle irradiation of the female are plotted in different ways in Figures 1a and 1b. In Figure 1a "Marker Presence" (the ratio of the number of recombinant cells of a particular class following X-irradiation of the female (N_R) to the number of cells of the input minority parent (N_I) is shown as a function of dose. The value of this ratio with no irradiation is proportional to the normal gradient function (e^{-kx}) and will be designated as N_{R0}/N_I. The survival (colony-forming ability) of the F^- cells is also shown. In Figure 1b the ordinate, "Marker Survival", is the ratio of Marker Presence following alpha irradiation of the F^- population to that with no irradiation, $\frac{N_R}{N_I}\Big/\frac{N_{R0}}{N_I} = N_R/N_{R0}$. Marker Presence and Marker Survival curves for both types of radiation are qualitatively similar.

Figure 1 shows that the Marker Presence and F^- survival curves converge with increasing dose delivered to the female while Figure 1b shows a divergence with dose of the Marker Survival curves from one another and from the F^- survival curve. Other points concerning the curves of Figures 1a and 1b are also pertinent: (1) the Marker Presence and Marker Survival of all markers decrease with irradiation dose and may be considered to be exponential within the experimental errors; (2) the slopes of these curves increase in absolute value in the inverse order of the distance of the selected markers from the origin; (3) the slope of the F^- survival curve is greater than the slope of the Marker Presence or Marker Survival curve of any marker; and (4) X rays and alpha particles have qualitatively equivalent biological effects although quantitatively a given dose of alpha particles is about 1.7 times as effective as the same dose of X rays.

The recombinational rate for an Hfr marker located at a distance x from the original can be written as Ce^{-kx}, where C is the recombinational rate of a marker located at the origin of the male chromosome and k, the overall gradient, is a constant for the specific mating conditions used. This overall gradient may be considered to be the sum of the gradient of transfer and the gradient of integration and segregation. With unirradiated cells, the gradient of transfer is larger than the gradient of integration and segregation; this latter is often assumed to be negligible. It can be seen from the convergence of the curves in Figure 1a that at higher doses there is a relative enhancement in the appearance of the distal markers compared to the proximal ones, that is, the overall gradient k is decreased. This can be confirmed by plotting the logarithm of marker presence against x, marker position, for various doses. Female irradiation would not be expected to decrease the gradient of transfer. Therefore, the observed decrease in the overall gradient can be associated with radiation effects on those recombinational events that follow transfer, that is, on the integrative and segregational

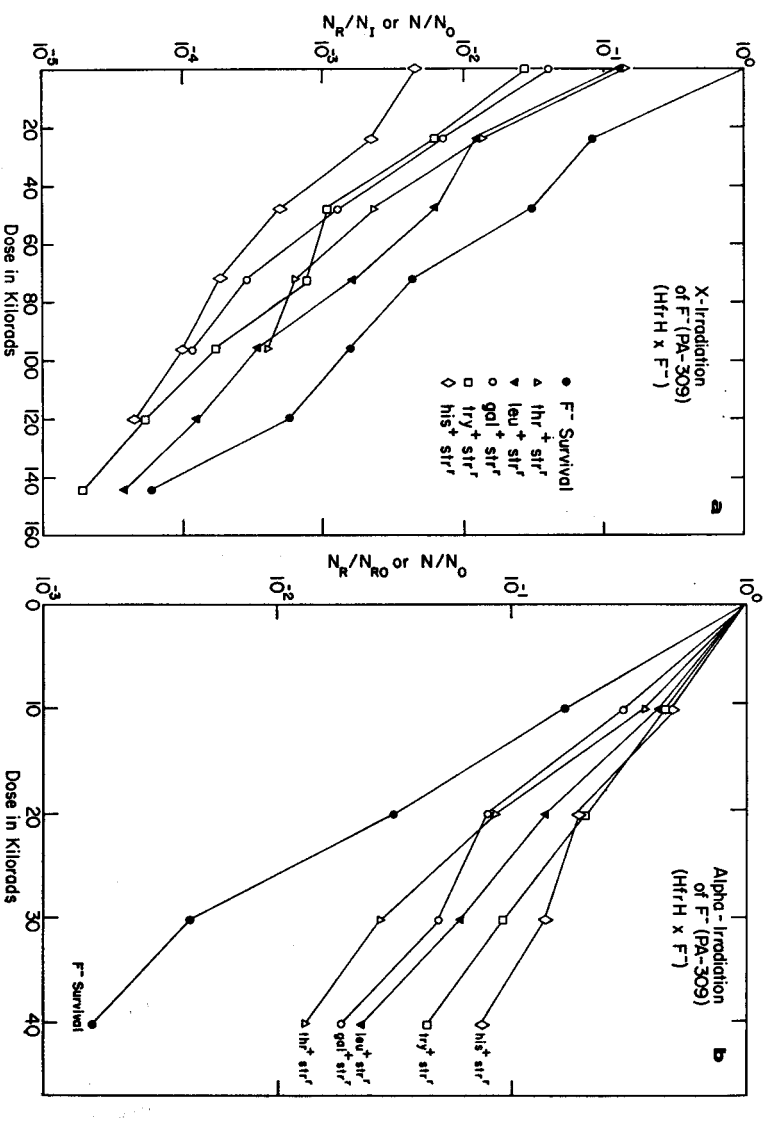

FIGURE 1.—Effect of X rays (a) and alpha particles (b) on the recombination frequencies for selected markers in a cross between a non-irradiated male and an irradiated female. *E. coli* Hfr H and *E. coli* F⁻ PA-309 are grown in broth to a concentration of 2 to 3×10^8 cells/ml. The females are irradiated on millipore filters with different doses of either X rays or alpha particles. After removal from the filters cell survival is assayed by scoring ability to form colonies on minimal medium ("Survival"). Aliquots of the irradiated females for each dose are mated with males (3×10^8 F⁻ and 2×10^7 Hfr cells/ml) for 90 min at 37°C with gentle agitation. Aliquots for each dose are plated and grown for 48 hr at 37°C on the proper media to determine the various recombinant classes. Counter selection is by streptomycin. In "a" the ordinate is "Marker Presence" (the ratio of the number of recombinant cells of a particular class following X-irradiation of the female (N_R) to the number of cells of the input minority parent (N_I)). In "b" the ordinate is "Marker Survival" (the ratio of "Marker Presence" following alpha-irradiation of the F⁻ population (N_R/N_I) to that with no irradiation (N_{RO}/N_O), or N_R/N_{RO}). "Survival" in both graphs refers to the ratio of F⁻ cells sur-

processes. In these experiments, these processes involve an undamaged portion of the male chromosome and the radiation damaged female chromosome. The more distal the selected marker, the larger the segment of the male chromosome that is known to be within the zygote. These results suggest that the presence of a portion of the male chromosome may prevent lethal expression of radiation-induced lesions on the corresponding portion of the female chromosome. Thus the apparent negative gradient of integration and segregation observed after female irradiation can be viewed as due to a selective advantage conferred on those recombinants which receive larger portions of the male chromosome.

The convergence of the marker survival curves at high doses (Figure 1a) can be visualized to result from a preferential utilization of the male genetic information. At high dose levels where the female chromosome may be badly damaged, viable recombinants may receive their genetic information almost entirely from the male chromosome.

Effect of irradiation on inheritance of unselected markers: The graphs in Figure 2 give the fraction of the recombinant population prototrophic for a selected marker and resistant to streptomycin that is also prototrophic for a second marker not originally selected for in the cross (the unselected marker) as a function of radiation dose received by the female. A selected marker operationally divides the male chromosome into two regions: the region between it and the origin (the anterior region) and the other part of the chromosome (the posterior region). Several generalizations can be made from the data: (1) X rays and alpha particles are qualitatively and approximately quantitatively equivalent in their effects on the inheritance of unselected markers. (2) In general, an unselected anterior marker appears with a frequency of 0.5 ± 0.1 in a cross involving an unirradiated F population and its frequency increases with dose to the 0.8 ± 0.1 level. No change is observed for the closely linked markers threonine and leucine (0.5 minute apart) which show a linkage of about 90 percent even at high dose. (3) The frequency of appearance of an unselected posterior marker rises from a value characteristic of the distance between the two markers (e^{-kx}) to a much higher value (between 0.5 and 0.9).

Thus in both the anterior and posterior regions there is a general increase in correlation of the selected and the unselected markers with increasing dose. This high level of increase of correlation is consistent with the data of Figure 1 and leads to the same conclusion, *i.e.*, those zygotes that have received larger pieces of the male genetic material have a better chance of producing viable recombinants.

DISCUSSION

The effects of either X-ray or alpha-particle irradiation of the recipient cell before mating can be briefly summarized: (1) the radiosensitivity for marker appearance or marker survival is inversely related to the distance between the origin of the male chromosome and the position of the selected marker (Figure 1); and (2) there is a preferential utilization with increasing dose of male genetic information in surviving recombinant cells (Figure 2).

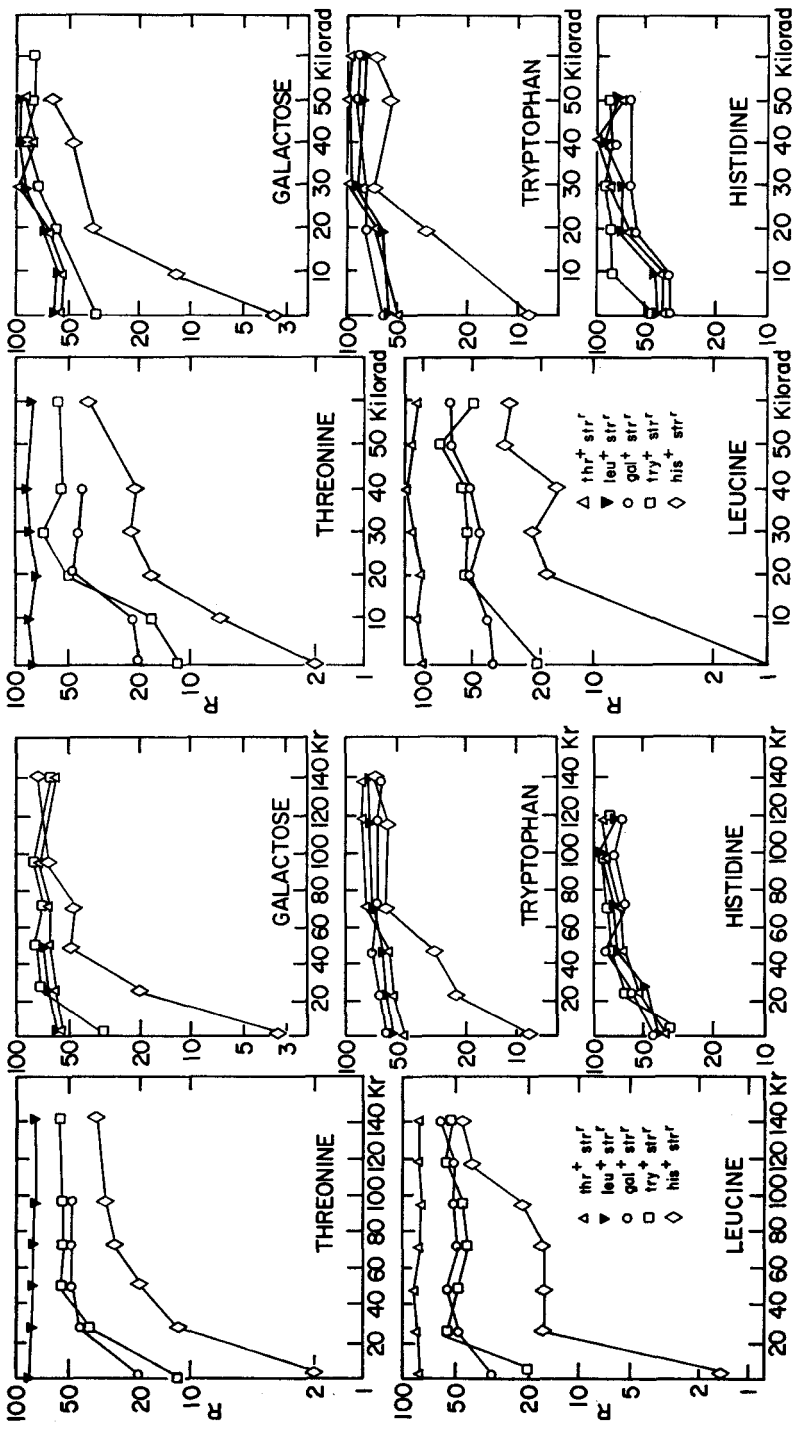

FIGURE 2.—Effect of X rays (a) and alpha particles (b) on the presence of unselected markers in a population of recombinants selected for a specific character. The recombinant colonies appearing in the experiment described in Figure 1 are reisolated and tested for the presence of the other markers not selected for in the cross. The ordinate above is the percent of these colonies which contain also the unselected marker (R). The abscissa is the dose given to the female cell. From 100 to 200 colonies were tested for each point.

Two types of operational lesions may be envisaged to be produced in the female genome by irradiation: those affecting the relative contributions of genetic information from the two parental cells into the recombinant chromosome (switching or breakage lesions); and those which result in lethality when present in the recombinant chromosome as a result of either material incorporation (Breakage-and-Reunion) or copying (Copy-Choice). It should be noted that at the doses used in these studies mutations at the genetic loci utilized are several orders of magnitude less frequent than the effects on the recombinational processes considered here.

The two simple models that have been proposed for genetic recombination predict somewhat different dose-response patterns for these two types of lesions:

(1) *Copy-Choice:* Switching lesions, as suggested by JACOB and WOLLMAN (1958), could cause the copying to be selectively switched away from the irradiated female template to the male one, leading thereby to a progressive utilization with dose of genetic information from the donor cell. Lethal lesions could also bring about selection for recombinants having primarily the information from the male genome because of linkage between the female characters and the radiation lethals on the recipient chromosome. Thus either type of lesion would result in an apparent increase in linkage between male characters in the recombinants with increasing radiation dose (Figure 2).

(2) *Breakage-and-Reunion*: It is known in higher organisms that ionizing radiations produce chromosomal breaks which may rejoin; it may be expected that such events occur in irradiated bacteria. If only breakage-type lesions are induced by the irradiation and if they act to produce recombinant types in the same manner as physiological breaks (*i.e.*, through a symmetric exchange of genetic material), male markers showing linkage with no irradiation (*e.g.*, thr^+ and leu^+) would become less linked with increasing radiation dose received by the recipients while more distant, unlinked markers (*e.g.*, thr^+ and his^+) would remain unlinked; thus a preferential utilization of the male genetic determinants would not be observed. On the other hand, if only lethal lesions are produced, there would be linkage between them and the female determinants. This could lead to a preferential selection for recombinants inheriting primarily the male markers, a result consistent with the data of Figure 2. If both types of lesions are present, an increased lingage of the male markers with dose would occur only if the rate of induction of lethal lesions is greater than the rate of induction of breakage ones, a more demanding condition than that imposed by a simple copy-choice mechanism.

More complicated models, such as the "Breakage-and-Copy" one suggested by MESELSON and WEIGEL (1961) for recombination in phage, may also be consistent with these data.

A detailed mathematical treatment of these results will appear elsewhere.

SUMMARY

In genetic recombination involving crosses between *Escherichia coli* K-12 donors (HfrH) and recipients (PA-309), the effects of X-ray or alpha irradia-

tion of the recipient before mating have been studied. The radiosensitivity for the appearance of a selected male marker in a recombinant cell is inversely related to the distance between the origin of the male chromosome and the position of the marker. Also, the linkage between male markers in the recombinants increases with dose received by the female. These results suggest that a selective advantage is conferred on those recombinants which receive larger portions of the male chromosome during mating and that at high dose levels where the female chromosome may be badly damaged, recombinants receive their genetic information almost entirely from the male chromosome. The results can be easily interpreted by a Copy-Choice mechanism for genetic integration in which radiation lesions act as switching sites, but are not inconsistent with a Break-and-Reunion model in which linkage between lethal radiation lesions on the female chromosome and the female characters provides a bias in favor of male inheritance.

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