MODIFICATION OF CONJUGATION IN *ESCHERICHIA COLI* K-12 BY ULTRAVIOLET IRRADIATION[1]

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CONJUGATION in *Escherichia coli* K-12 results from a sequence of consecutive steps: (1) an effective *union* between two cells of opposite mating types; (2) a polarized *transfer* of at least a portion of a male chromosome into the recipient cell, the precise order of transfer depending only on the donor strain utilized; (3) an *integration* of the genetic information from both parental strains into a recombinant chromosome; and (4) the *segregation* of the recombinant chromosome from the residual genetic information within the zygote with the subsequent production of a recombinant clone (see JACOB and WOLLMAN 1961; CLARK and ADELBERG 1962, for reviews).

In previous studies the effects of ionizing radiations delivered before mating to either donor or recipient cells have been described (MARCOVICH 1961; WOOD and MARCOVICH 1964). Briefly, irradiation of the male produces lesions which prevent the transfer of the male chromosome to the female; no radiation lesions are produced which hinder the ability of the cells to form an effective union and no detectable effects on the processes of integration and segregation have been observed. The appearance of a specific male marker decreases exponentially with the dose to which the donor cells are exposed, and the rate of loss of appearance increases with the distance of a particular male marker from the Origin (the portion of the male chromosome which first enters the female). It is possible to construct chromosome maps using radiosensitivity as an index of marker position that are identical to those generated by the interrupted mating technique (JACOB and WOLLMAN 1961).

Irradiation of the recipient cells, on the other hand, does not affect detectably the transfer step but affects primarily the steps of union and integration. In the production of recombinants, there is a selective utilization of genetic information from the nonirradiated male parent as if there were an advantage conferred on those zygotes that receive larger portions of the male chromosome during mating. This results in an apparent increase in linkage between the male markers.

JACOB and WOLLMAN (1961) have studied the effects of ultraviolet (UV) irradiation on conjugation in the K-12 mating system. They have reported that:

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(1) the number of recombinants having a specific male marker decreases exponentially with the UV dose received by the male parent; (2) the rate of decrease is approximately the same for all of the male markers studied; (3) UV irradiation of the male parent before mating decreases the apparent linkage between markers contributed by the donor in the recombinant cells; and (4) UV irradiation of the female parent before mating decreases the apparent linkage between markers contributed by the donor in the recombinant cell. Our studies to be reported in this paper confirm (1), only partially confirm (2) and (3) and are not in accord with (4). We will show that UV irradiation affects several of the steps of conjugation and that it is possible to separate these by graphical and mathematical analysis.

MATERIALS AND METHODS

Strains: Escherichia coli K-12. *Recipient:* F⁻ PA-309 (*thr*⁻, *leu*⁻, *try*⁻, *his*⁻, *gal*⁻, *str*^{r}) where the abbreviations symbolize threonine, leucine, tryptophan, histidine, galactose and streptomycin, respectively. The gene symbols as written indicate that the strain shows dependence for the various amino acids or cannot use galactose as an energy source; s and r indicate sensitivity or resistance of the strain to an agent. *Donor:* HfrH (*str*^{s}). Interrupted mating experiments with these strains (Low and Wood 1965) give the following minimum times required for the transfer of the pertinent markers from the male to the zygote: *thr*+: 8 minutes; *leu*+: 8.5 min; *gal*+: 24 min; *try*+: 33; and *his*+: 54 min. These minimum transfer times include a mobilization time (Clark and Adelberg 1962) that is approximately 4 minutes (Low 1965); accordingly the distances of the markers from the Origin (Marker Distance) are: *thr*+: 4 min; *leu*+: 4.5 min; *gal*+: 20 min; *try*+: 29 min; and *his*+: 50 min. Streptomycin sensitivity is transferred with very low frequency to the zygote under the conditions used here.

Media: These are identical to those used in earlier studies (Wood and Marcovich 1964).

Mating and assay: Male and female cells were normally grown in tryptone broth to concentrations of 2 to 3×10^8 cells/ml (exponential phase). The parent to be irradiated was washed and resuspended in 0.9% saline in order to minimize UV absorption by the medium; however, neither growth nor resuspension in minimal medium modified any of these results. Aliquots of male and female cells totaling 2 ml were mixed together for mating and gently agitated at 37°C for 90 minutes. Generally the concentration of the irradiated parent was 1/10 of that of the nonirradiated one although variations between 1/20 and 20/1 in the mating ratios do not affect the results reported here. All calculations are made with respect to the input number of the minority parent in the mating suspension.

To determine cell survival and recombinant production, aliquots from the mating suspension 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 onto various test plates.

Irradiation: A 10 ml sample of cells was placed in an open petri dish at a distance of 50 cm from a horizontal, 15-watt germicidal lamp (G.E.) emitting primarily at 2537Å. The incident flux was monitored before each experiment and adjusted to 25 ergs/mm^2/sec. Extreme precautions were taken to avoid any photoreactivation.

Each experiment reported has been repeated at least three times. Normally duplicate platings are done for each assay point; standard errors are indicated on the following graphs. Additional information on the experimental procedures is given in the legends for the figures.

RESULTS

A. *Irradiation of the male:* In Figure 1 the ratio of the number of recombinants

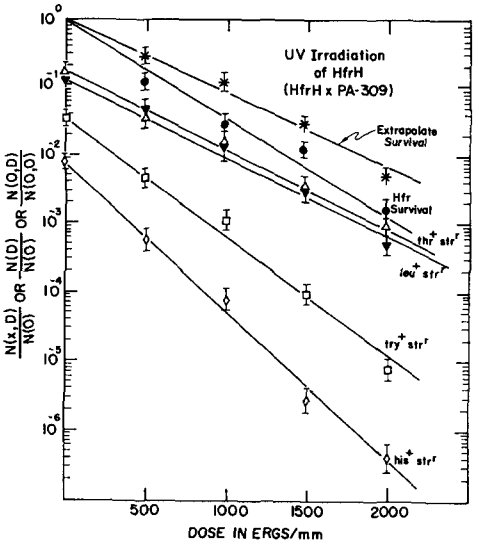

FIGURE 1.—Effect of UV irradiation on the recombination frequencies for selected markers in a cross between an irradiated male and a nonirradiated female. *E. coli* HfrH and F- PA-309 are grown in liquid medium to concentrations of about 2×10^8 cells/ml. The males are resuspended in saline and UV irradiated. Aliquots of the irradiated males at each dose level are mated with females (2×10^8 F- and 2×10^7 Hfr cells/ml) for 90 minutes at 37°C with gentle agitation. Aliquots for each dose are plated and grown for 48 hours at 37°C on the proper media to determine the various recombinant classes. Counter selection is by streptomycin. Survival of males is assayed by scoring their ability to form colonies on minimal media. The ordinate is Marker Presence (the ratio of the number of recombinant cells of a particular class following UV irradiation of the male ($N(x,D)$) to the number of male cells in the mating suspension ($N(0)$), or $N(x,D)/N(0)$; or Hfr Survival $N(D)/N(0)$. The abscissa is the UV dose delivered to the male cells. See text for additional details.

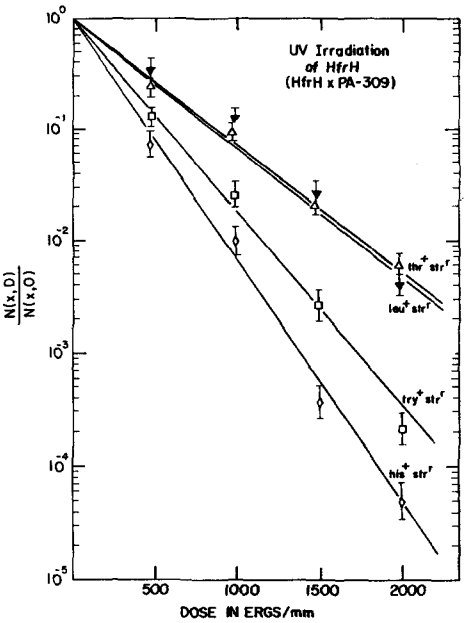

FIGURE 2.—Effect of UV irradiation of the donor parent on Marker Survival $N(x,D)/N(x,0)$. The experimental conditions are described in the legend for Figure 1.

having a selected male marker located at a distance x from the Origin to the number of male cells in the mating suspension [$N(x,D)/N(0)$ or "Marker Presence"] is plotted as a function of the UV dose D delivered to the male population before mating. The male survival ratio $N(D)/N(0)$ is also shown. Three points are to

be noted: (1) the curves are linear (i.e., exponential) within the limits of the experimental errors; (2) $N(x,0)/N(0)$ (the intersection of these curves with the $D = 0$ axis) decreases with the distance of the marker from the origin; and (3) the slopes for the various markers increase with the distance of the selected marker from the origin. In their study, JACOB and WOLLMAN (1961, p. 238) investigated only markers between the origin and galactose with doses below about 1000 ergs/mm^2 (our estimate) and reported that the various marker slopes were about the same. The difference in slopes is more clearly shown in Figure 2 in which "Marker Survival," $\frac{N(x,D)}{N(0)} \Big/ \frac{N(x,0)}{N(0)}$ or $N(x,D)/N(x,0)$, is shown as a function of dose. Notice that the slopes increase with marker distance, but that they are clearly not proportional to the distances of the various markers from the origin as has been found for ionizing radiations (MARCOVICH 1961; WOOD and MARCOVICH 1964; KRISCH 1965). (The ratio of the distance from the origin to the most distal marker used here, *his*$^+$, to that for the closest marker, *thr*$^+$, is about 12.) Accordingly it is not possible to use these UV data in their present form to construct proportional chromosome maps.

In Figure 3 the dependence of Marker Presence, $N(x,D)/N(0)$, on marker position, x, is shown for various doses (gradient plots). These curves are also linear and show slopes that increase with UV dose. It can be seen that extrapolates of these curves to zero marker distance (or a marker entry time of about 4 minutes) intercept the Origin axis at values $N(0,D)/N(0)$ which decrease with higher doses. Similar plots for X-ray or alpha particle irradiation or P^{32}

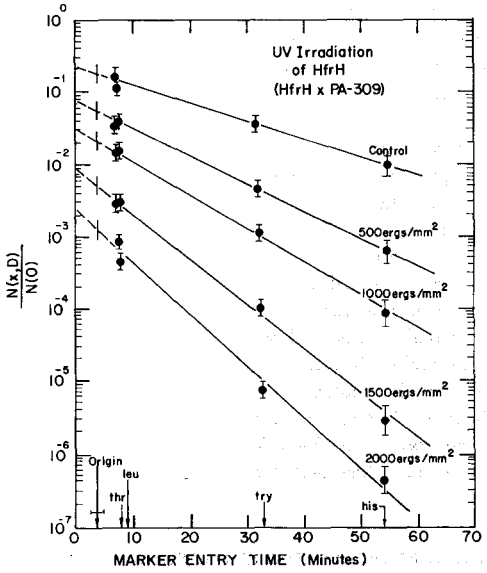

FIGURE 3.—Effect of UV irradiation of the donor parent on the gradient. Marker Presence $[N(x,D)/N(0)]$ is plotted as a function of marker entry time for various doses. The experimental conditions are described in the legend for Figure 1.

decay in the male do not show this property for the extrapolates—for these treatments the extrapolates for all doses pass through a single point on the vertical axis (Wood and Walmsley, unpublished). Such extrapolates are related to the probability that a marker immediately adjacent to the origin will appear in the recombinant population. It will be shown later that this decrease in the extrapolates with dose may be due to a decrease in the ability of irradiated male cells to form effective unions. To test this hypothesis, irradiated male cells were pulled down onto multiple layers of female cells supported on membrane filters (0.45 microns spore size) so as to bring cells of opposite mating types into juxtaposition; the filters were transferred to the surface of a prewarmed tryptone broth plate and matings were allowed to proceed (Matney and Achenbach 1962). The results differed from those of Figure 3 in two ways: (1) recombinants in the various classes for no irradiation were about twice as numerous as when matings were in liquid broth; and (2) the extrapolate values decreased by about a factor of 10 as the dosage increased from 0 to 2000 ergs/mm^2 for the membrane matings as contrasted with a variation of about 100 for matings in liquid broth.

The graphs in Figure 4 (the genetic analysis) give the fraction of the population prototrophic for a selected marker located at a distance x from the Origin and resistant to streptomycin that is also prototrophic for a second marker located at a distance y from the origin not originally selected for in the cross (the un-

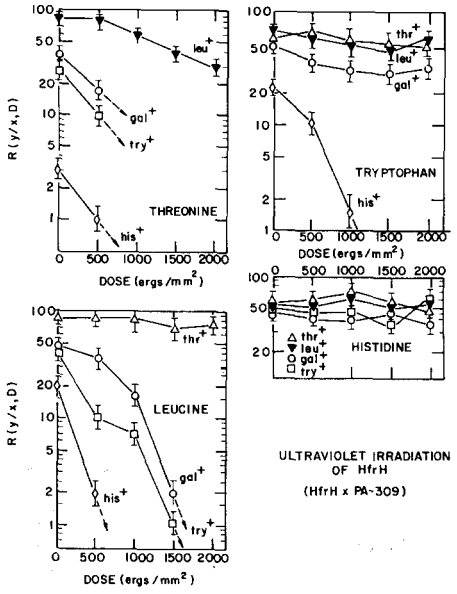

Figure 4.—Effect of ultraviolet irradiation of the male on the presence of unselected markers in a population of recombinants selected for a particular character. The recombinant colonies appearing in the experiment described in Figure 1 are reisolated and tested for the presence of other markers not selected for in the cross. The ordinate above ($R(y/x,D)$, see text) is the percent of these colonies which contain also the unselected marker. The abscissa is the dose given to the donor cells. From 200 to 400 colonies were tested for each point.

selected marker), $R(y/x,D)$, as a function of UV dose D received by the donor parent. Unselected markers may be divided conveniently into two categories: (1) those that are between the origin and the selected marker (proximal); and (2) those that are distal to the selected marker (distal). Two generalizations can be made from these data: (1) the appearance of a distal, unselected marker decreases with dose and the rate of decrease is greater the more distant the unselected marker (e.g., the unselected markers *gal*$^+$, *try*$^+$, and *his*$^+$ among *leu*$^+$ recombinants); (2) the appearance of a proximal, unselected marker is only slightly, if at all, modified by UV irradiation (e.g., all unselected markers among *his*$^+$ recombinants). From less complete data (only one UV dose, and fewer widely spaced markers), JACOB and WOLLMAN (1961, p. 239) reported that both proximal and distal unselected markers appeared less frequently after male irradiation. Our data do not confirm their results for proximal, unselected markers. Furthermore, the decreased appearance of unselected distal markers is not due primarily to decreased linkage between male markers but is due to a diminution in the probability of transfer of the unselected marker with increasing radiation dose (see DISCUSSION).

B. *Irradiation of the female:* Ratios of the numbers of recombinants having a

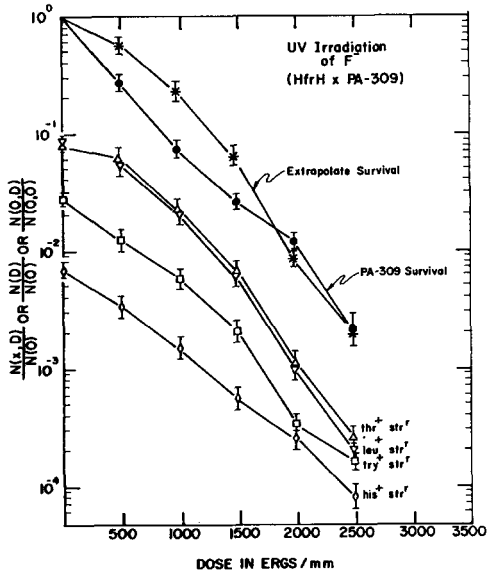

FIGURE 5.—Effect of ultraviolet irradiation of the recipient parent on Marker Presence (N(x,D)/N(0). The experimental conditions are the same as described in Figure 1 except that the female parent is irradiated and is the minority parent in the mating suspension (2×10^8 Hfr and 2×10^7 F$^-$ cells/ml). The Extrapolate Survival and the F$^-$ Survival are also given. The abscissa is the UV dose delivered to the female cells.

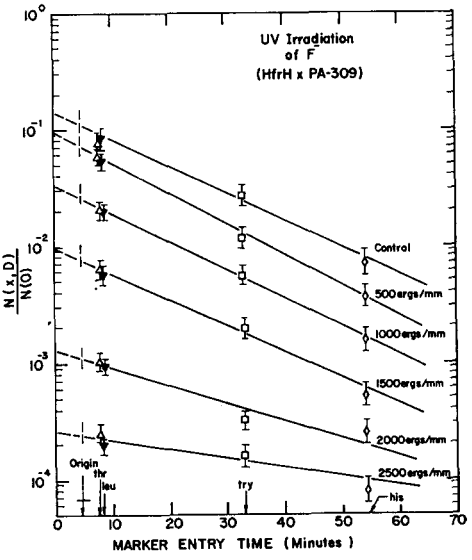

FIGURE 6.—Effect of ultraviolet irradiation of the recipient parent on the gradient. Marker Presence is plotted as a function of marker position for various doses. The experimental conditions are described in the legend for Figure 5.

selected male marker to the number of female cells in the mating suspension ($N(x,D)/N(0)$, or Marker Presence) are plotted in Figure 5 as a function of UV dose delivered to the female population before mating. The female survival $N(D)/N(0)$ is also shown. The female survival curve is exponential with dose and the Marker Presence curves all decrease with dose; contrary to the results obtained with male irradiation, the slopes of the Marker Presence curves vary inversely with the distances of the male markers from the Origin. This leads to an apparent convergence of the Marker Presence curves at higher doses. Gradient plots ($N(x,D)/N(0)$) for different doses are given in Figure 6 as functions of male marker position. Again, contrary to the results obtained with male irradiation, the slopes of the gradient plots decrease for higher doses such that at the higher doses all of the male markers appear in recombinants with approximately the same frequencies. The extrapolates to these curves [$N(0,D)/N(0)$] are indicated; the Extrapolate Survival $N(0,D)/N(0,0)$ is also plotted in Figure 5.

The genetic analysis for female irradiation, $R(y/x,D)$, is given in Figure 7. For both proximal and distal unselected markers $R(y/x,D)$ increases with dose.

To test whether UV irradiation of the recipient parent affects chromosome transfer from the donor parent, interrupted mating experiments (Low and Wood 1965) were carried out with nonirradiated males and females exposed

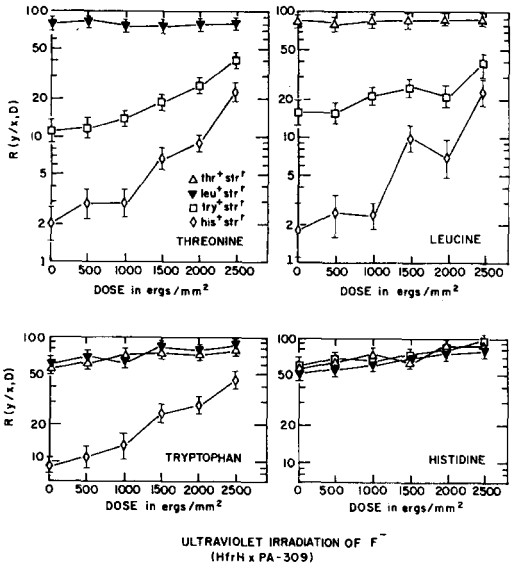

FIGURE 7.—Effect of ultraviolet irradiation of the female on the presence of unselected markers in a population of recombinants selected for a particular character. The recombinant colonies from the experiment of Figure 5 were analyzed. See the legend of Figure 4 for details.

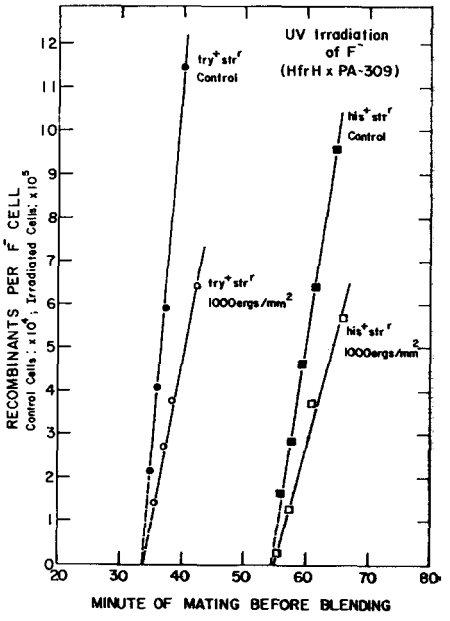

FIGURE 8.—Marker entry time for HfrH cells mated with nonirradiated females or with females previously exposed to 1000 ergs/mm² of UV irradiation. See Low and Wood (1965) for details on technique. The ordinate is 10× less for matings with irradiated females than with nonirradiated ones.

to 1000 ergs/mm^2. The entry times for the *try*$^+$ and *his*$^+$ markers are not measurably modified by this treatment (Figure 8); the number of recombinants for each entry time is reduced by this UV treatment by about a factor of 10, consistent with the results of Figure 5.

DISCUSSION

A. *Irradiation of the male:* Modification of bacterial conjugation by ultraviolet irradiation of the male before mating differs from that produced by ionizing radiations in at least two ways: (1) the survival curves for the various markers (Figure 2) do not have slopes that are proportional to marker distances; and (2) the extrapolates for the gradient plots for various doses (Figure 3) do not pass through a single point. Both of these differences indicate that UV effects may be more complex than those produced by ionizing radiations.

Recombinant production can be analyzed in terms of the four consecutive and independent steps of union, transfer, integration and segregation. Only the transfer step should be a function of marker position x, although all four steps could be affected by irradiation of the donor parent (Wood and Walmsley, unpublished). Since the four steps are independent we write

$$N(x,D) = N(0) \cdot P_u(D) \cdot P_t(x,D) \cdot P_i(D) \cdot P_s(D) \qquad (1)$$

where $N(x,D)$ is the number of recombinants that are streptomycin resistant and prototrophic for a male marker located a distance x from the origin, $N(0)$ is the number of the minority parent (here, the male) in the mating suspension, and $P_u(D)$, $P_t(x,D)$, $P_i(D)$ and $P_s(D)$ are the independent probabilities for the consecutive steps of union, transfer, integration and segregation, respectively. It is convenient to write $N(x,D)$ as the product of a function that is dose dependent only and one that is both dose and position dependent:

$$N(x,D) = N(0) \cdot A(D) \cdot P_t(x,D), \qquad (2)$$

where

$$A(D) = P_u(D) \cdot P_i(D) \cdot P_s(D) \qquad (3)$$

With no irradiation it is found experimentally (Figure 3, $D = 0$ curve) that

$$N(x,0)/N(0) = A(0)\, e^{-\nu^0_{mt} x} \qquad (4)$$

where $A(0)$ and ν^0_{mt} are constants for a particular set of mating conditions. This experimental result is consistent with the idea that the positional dependence of marker appearance resides completely in the transfer probability and is due to random events in the male chromosome that prevent transfer. (If the probability of such an event per unit length is ν^0_{mt}, then there would be on the average $\nu^0_{mt} x$ events between the Origin and a male marker at x; the probability of having no events in this region is the first term of the Poisson distribution, or $e^{-\nu^0_{mt} x}$.)

When male cells are exposed to ionizing radiation (Marcovich 1961; Krisch 1965; Wood and Walmsley, unpublished), the experimental results are described analytically by

$$N(x,D) = N(0)A(0)e^{-(\nu^0_{mt} + \sigma_{mt} D)x} \quad \text{<ionizing radiation>} \qquad (5a)$$

that is

$A(D) = A(0)$ and $P_t(x,D) = e^{-(\nu^0_{mt} + \sigma_{mt} D)x}$ <ionizing radiation> (5b)

These results are consistent with the idea that ionizing radiations produce additional lesions preventing transfer randomly along the chromosome at a constant rate, σ_{mt}.

For our analysis of the effects of UV irradiation we define the function "Extrapolate Survival" as $\frac{N(0,D)}{N(0)} \Big/ \frac{N(0,0)}{N(0)} = N(0,D)/N(0,0)$; values for $N(0,D)/N(0)$ and $N(0,0)/N(0)$ for various UV doses can be determined from the curves of Figure 3 (the extrapolates) and the Extrapolate Survival can be computed. These are plotted in Figure 1 as a function of dose. This linear dependence allows us to write

$$N(0,D)/N(0,0) = e^{-\beta D} \tag{6}$$

where β is an experimental constant. Using equation (2) twice, the identities $P_t(0,D) = 1$ and $P_t(0,0) = 1$ and equation (6), we have

$$N(0,D)/N(0,0) = A(D)/A(0) = e^{-\beta D} \tag{7}$$

From the experimental results of Figure 21, we may write

$$N(x,D)/N(x,0) = e^{-f(x)D} \tag{8}$$

where $f(x)$ is the slope of the curve for the male marker at x. We next form the ratio $\frac{N(x,D)}{N(x,0)} \Big/ \frac{N(0,D)}{N(0,0)}$ using the proper experimental values of the slopes (Figure 2); plots of this function for various values of x are given in Figure 9 as a function of dose. These normalized curves are again exponential with slopes $F(x)$ and we write accordingly by use of equations (6) and (8):

$$\frac{N(x,D)}{N(x,0)} \Big/ \frac{N(0,D)}{N(0,0)} = e^{-(f(x)-\beta)D} = e^{-F(x)D} \tag{9}$$

A plot of the experimental values of $F(x)$ (the slopes of the curves of Figure 9) against x, gives a linear relation (Figure 10); accordingly, we have

$$F(x) = f(x) - \beta = \sigma_{mt} x \tag{10}$$

where σ_{mt} is an experimental constant. Lastly we rewrite and rearrange equation (9) using equations (4), (6) and (10):

$$N(x,D) = N(0)\, A(0) e^{-\beta D}\, e^{-(\nu^0_{mt} + \sigma_{mt} D)x} \tag{11}$$

This has the form of equation (2) where

$$A(D) = A(0)e^{-\beta D} \text{ and } P_t(x,D) = e^{-(\nu^0_{mt} + \sigma_{mt} D)x} \tag{12}$$

Note that equation (11) is identical in form to equation (5a) if $\beta = 0$. Furthermore, the linearity between the slopes of the corrected marker survival curves and marker position (Figure 10) makes possible the construction of proportional chromosome maps from the determinations of ultraviolet sensitivities of the various markers.

Consider next the genetic analysis (Figure 4). Our results differ considerably from those previously reported by JACOB and WOLLMAN (1961, p. 239) in which they utilized only a limited region of the male chromosome and one or a few doses of UV irradiation. They have reported that the apparent linkage between male markers was decreased when the donor bacteria were UV treated. The data

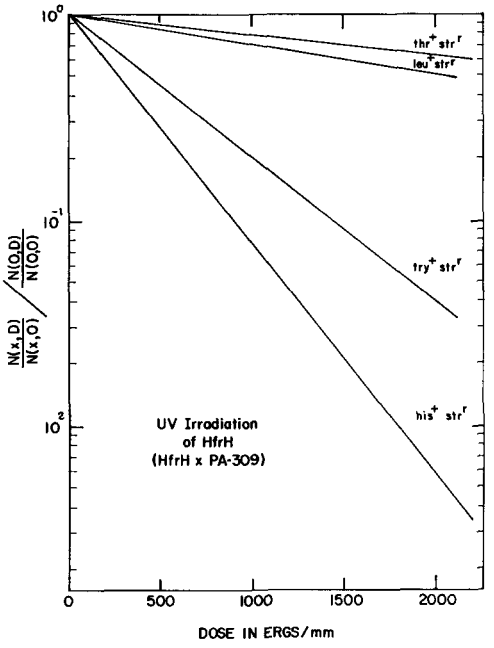

FIGURE 9.—Corrected Marker Survival curves or $\frac{N(x,D)}{N(x,0)} \Big/ \frac{N(0,D)}{N(0,0)}$. The curves of Figure 2 have been corrected for Extrapolate Survival (see text).

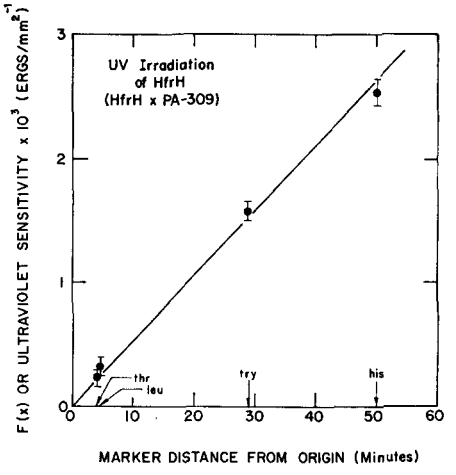

FIGURE 10.—The dependence of the slopes of the Corrected Marker Survival Curves (Figure 9) on marker position.

in Figure 4 are qualitatively identical to those obtained earlier with X rays (MARCOVICH 1961), with alpha particles (WOOD and WALMSLEY, unpublished), and with decay of P^{32} incorporated in the male (KRISCH 1965) and may accordingly be analyzed by the methods used earlier. If the unselected marker is proximal to the selected one (e.g., all other markers when selection is for *his*$^+$), $R(y/x,D)$ is dose independent and has the value of about 0.5 for markers that are widely separated. For markers less separated (e.g., *thr*$^+$ when selection is for *leu*$^+$), the value is considerably higher, about 0.85. In these cases $R(y/x,D)$ gives the linkage between the two markers and is dose independent. Therefore, no detectable lesions are induced at moderate doses which are carried on the transferred male chromosome and subsequently modify integration or segregation.

If the unselected marker is distal to the selected one (e.g., all other markers when selection is for *thr*$^+$), $R(y/x,D)$ is dose dependent and decreases with both distance between the markers $(y - x)$ and with dose. This decrease is due to the dependence of the transfer probability $P_t(y - x,D)$ on distance and dose (WOOD and WALMSLEY, unpublished). In this situation, therefore, it is improper to speak of linkage in the classical sense because the results may be grossly distorted by a diminution with dose of the transfer probability. If proper corrections are made

for the transfer, a linkage factor can be obtained even for distally located male markers, i.e., $L(y-x) = R(y/x,D)/P_t(y-x,D)$. These experiments with UV irradiation of the male do not indicate a dependence of the linkage on dose.

From the above results we infer that two types of lesions pertinent to conjugation are induced by ultraviolet irradiation of the donor .The first type, equivalent in its action to the type produced by ionizing radiations, results operationally in an inability of the male chromosome to be transferred into the female. In the now classical theory of JACOB and WOLLMAN (1961), the male chromosome is postulated to be pushed in some unknown fashion into the recipient cell with no concomitant DNA synthesis. A break introduced prior to mating in the male DNA by radiation, or a lesion which results operationally in a break during transfer, would prevent the transfer of markers distal to this breakage point. This model is compatible with the data presented here and elsewhere in which radiations are used to modify transfer (FUERST, JACOB and WOLLMAN 1956; MARCOVICH 1961; KRISCH 1965). Ultraviolet irradiation is known to cause chromosomal breaks in higher organisms (see WOLFF 1961, for summary) and could act similarly in this system.

In the more recent theory for chromosome transfer proposed by JACOB, BRENNER and CUZIN (1963), it is assumed that an effective union between the donor and recipient cell is followed by DNA synthesis starting at the Origin with the accompanying transfer of one of the newly formed hybrid chromosomes into the female. It is reasonable to assume that a break or a lesion in the template DNA would either interrupt synthesis or modify it in such a way that the transfer would be halted at the site of the radiation insult. This model, in the present context, is operationally equivalent to the first.

In a recent study DOUDNEY and BRUCE (1966) report a correlation between recovery of ability to produce recombinants and DNA synthesis in Hfr cells exposed to low doses of ultraviolet irradiation before mating. Post-irradiation recovery of this nature was found to be prevented by metabolic inhibitors or conditions which block DNA synthesis. They interpret their results as supporting the JACOB, BRENNER and CUZIN model.

The second type of lesion produced by UV (the position independent component or Extrapolate Survival, $A(D)/A(0) = e^{-\beta D}$) can be associated by this analysis with any of the steps of conjugation other than transfer. The only one of these that directly involves the irradiated male is union. It is reasonable to expect a relationship between the ability of a male cell to form an effective union and its ability to survive (colony formation) after UV treatment but the fact that the slope for the Extrapolate Survival curve is only about 70% of that for male survival makes this interpretation incomplete. Matings on membrane filters which maximize the juxtaposition of male and female cells decrease the slope of the Extrapolate Survival curve by about a factor of two; this indicates that at least half of the positional independent effect is operative on the step of union. The residual portion could affect the processes of mobilization which proceed transfer or the integration step. Modification of the integration step by radiation could be due to variations either in the juxtaposition of the two parental chromo-

somes prior to the integration of genetic information into a recombinant chromosome or in the integration process *per se*, or in both. Models for integration involving either copy-choice or break-and-rejoin mechanisms and radiations predict decreased linkage of the male markers with increasing dose (WOOD and MARCOVICH 1964), a prediction that is not verified by these experiments. We conclude, therefore, that a dose dependency of the integration step, if any, is affected through a modification in the juxtaposition of the parental chromosome.

B. *Irradiation of the female:* We have reported previously on the modification of genetic recombination by X-ray and alpha particle irradiation of the female (WOOD and MARCOVICH 1964) and by decay of radioactive phosphorus incorporated into the female population (KRISCH and WOOD 1965). The results from these studies and the present study involving UV irradiation of the recipient parent prior to mating are qualitatively quite similar. In all cases there is a convergence with dose of the Marker Presence curves (Figure 5), a flattening of the gradient curves with increasing dose (Figure 6), and an increase with dose in the values of $R(y/x,D)$ for both proximal and distal unselected markers (Figure 7). These results are quite divergent from those reported earlier by JACOB and WOLLMAN (1961).

When the recipient cells are irradiated, recombinants can be produced only if the fertilized zygote survives. This survival may be a function not only of the irradiation treatment but also of the availability of genetic material within the zygote which may be used in the production of a viable recombinant. In general, recombinant production is enhanced in those zygotes that receive larger amounts of male genetic information. At higher dose levels (low survival levels), recombinants show high linkage among all of the male markers (Figure 7) as if the male genetic information is preferentially used under these conditions in the production of recombinants. Thus two types of lesions may be produced in the recipient cell by irradiation: a type influencing the relative contributions of genetic information from the two parental cells into the recombinant chromosome (switching or breakage lesions); and a type that results in lethality when present in the recombinant chromosome either as a result of material incorporation (a break-and-rejoin mechanism) or DNA synthesis (a copy-choice mechanism).

If integration occurs only via a copy-choice mechanism, lesions induced on the female chromosome could act as switching sites causing 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 male parent. Lethal lesions on the female chromosome when copied could also bring about selection for recombinants having primarily male genetic information because of linkage between the female characters and radiation lethals on the female chromosome. Either type of lesion would result in an apparent increase in linkage between male characters in the recombinants, with increasing radiation dose.

On the other hand, if integration occurs only through a break-and-rejoin mechanism, radiation induced lesions which could result in breaks would serve to

effectively decrease the linkage between closely linked markers (e.g., *thr*$^+$ and *leu*$^+$); this is not found to happen (Figure 7). If lethal lesions are produced on the female chromosome by UV irradiation, linkage between female markers and these lethals would lead to a preferential selection for recombinants inheriting primarily the male markers, a result consistent with our data. If both types of lesions are produced, the rate of production of the second type must be greater than that for the first type. More complicated models for recombination may also be consistent with these data.

A more detailed mathematical model for all of these results will be given elsewhere.

SUMMARY

Ultraviolet irradiation before mating of either the donor parent (HfrH) or the recipient one (PA 309) greatly affects conjugation in *Escherichia coli* K-12. Ultraviolet irradiation of the male results in the production of at least two types of lesions: the first prevents the transfer of the male chromosome to the female, a result found previously with ionizing radiations; the second prevents the formation of an effective union between donor and recipient. When corrections are made for this second effect, it is possible to construct chromosome maps using ultraviolet sensitivity of the markers as an index of marker position that are identical to those generated by the interrupted mating technique. An apparent decrease in linkage between male markers following ultraviolet irradiation (JACOB and WOLLMAN 1961) is due to the first type of lesion and not to radiation effects operative at the integration level.

Irradiation of the female parent, on the other hand, does not affect detectably the transfer step but affects primarily the steps of union and integration. In the production of recombinants there is a selective utilization of genetic information from the nonirradiated male parent as if there were a selective advantage conferred on those zygotes that receive larger portions of the male chromosome during mating. This results in an apparent increase in linkage between the male markers.

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