CONJUGATION IN *ESCHERICHIA COLI* K-12 AND ITS MODIFICATION BY IRRADIATION

THOMAS H. WOOD *and* ROGER H. WALMSLEY

From the Department of Physics, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT The steps of normal bacterial conjugation (union, transfer, integration and segregation) are described in analytical terms. Only two parameters are utilized: ν_{mt}^0, the probability of interruption of transfer of the male chromosome per unit chromosomal distance; and ν_r^0, the probability per unit chromosomal distance of a recombinational event. Experimentally these two parameters have the same value (0.06 min^{-1} or 10^{-6} per nucleotide pair). Irradiation of the *donor* parent prior to mating increases the transfer parameter ($\nu_{mt} = \nu_{mt}^0 + \sigma_{mt}D$) and a complete description of the radiation response of recombinant production is obtained by a consideration of the single parameter σ_{mt}. Irradiation of the *recipient* parent prior to mating increases the recombination parameter ($\nu_r = \nu_r^0 + \sigma_r D$) and a complete description of the radiation response of recombinant production is obtained by the addition of the parameter σ_r. Experimentally σ_{mt} and σ_r are found to have the same value, approximately 0.004 $krad^{-1}$ min^{-1} for X-irradiation. It is thus possible to describe mathematically the behavior of the unperturbed mating system by a single parameter ν^0; a single additional parameter σ is adequate to describe the behavior of the system when either parental type is irradiated prior to mating. The unexpected observation that ν_{mt} and ν_r have the same value suggests that common molecular mechanisms are involved in the transfer and integration steps.

I. INTRODUCTION

In a series of fundamental investigations Hayes (1953) and Jacob and Wollman (1961) have shown that conjugation in *Escherichia coli* K-12 consists of a series of consecutive steps: (1) a pairing between a donor cell (Hfr) and a recipient one (F^-) which produces an effective *union*; (2) the *transfer* of a portion of a donor chromosome into the recipient cell in an ordered, sequential fashion that depends only on the particular donor strain utilized; and (3) the *integration* of the genetic information thereby transferred and that of the recipient parent into a recombinant chromosome which is subsequently *segregated* from those of the female parent. The over-all process of conjugation and these individual steps have been discussed in reviews by Clark and Adelberg (1962) and by Gross (1963).

Perturbations, in particular ionizing radiations, applied to either parent at dif-

ferent times during conjugation have been found to modify recombination (Marcovich, 1961; Wood and Marcovich, 1964; Krisch, 1965; Krisch and Wood, 1965; Joset and Wood, 1966) and considerable information on the individual steps of the process may be obtained from such studies. The purpose of this paper is threefold: (1) to present additional experimental information on the effects of radiations on these processes; (2) to summarize some of the quantitative data that are available on these steps; and (3) to provide a model for conjugation in this system and its modification by radiations and other injurious agents. Although our conclusions are based completely on studies with the *Escherichia coli* K-12 mating system using primarily the donor strain originally isolated by Hayes (HfrH), this formalism for bacterial recombination may have fairly general validity.

II. MATERIALS, METHODS, AND SYMBOLISM

Strains. Escherichia coli K-12. *Recipients.* PA-309 (*thr*$^-$, *leu*$^-$, *try*$^-$, *his*$^-$, *gal*$^-$, *str*^{r}) and PA-330 (*thr*$^-$, *arg*$^-$, *pro*$^-$, *str*^{r}), where the abbreviations are: thr, threonine; leu, leucine; try, tryptophan; his, histidine; gal, galactose; str, streptomycin; arg, arginine; pro, proline. The gene symbols as written indicate that the strain shows dependence on the various amino acids or cannot use galactose in lieu of glucose as an energy source; *s* and *r* indicate sensitivity or resistance to streptomycin. *Donors.* The minimum times in minutes at 37°C required for the transfer of pertinent markers from the male to the female are: HfrH (*str*^{s}): *thr*$^+$, 8; *leu*$^+$, 9; *gal*$^+$, 24; *try*$^+$, 36; *his*$^+$, 52. HfrCavalli (*str*^{s}): *pro*$^+$, 11; *thr*$^+$, 18, *arg*$^+$, 30.

Media. These have been described earlier (Wood and Marcovich, 1964).

Mating and Assay. Aliquots of male (Hfr) and female (F^-) cells grown in broth to concentrations of about 2×10^8 cells/ml (exponential phase) totaling 2 ml are mixed together and gently agitated at 37°C for various time intervals up to 90 min. Variations between 1:20 and 20:1 in the mating ratio (the ratio of Hfr to F^- cells in the mating mixture) do not affect significantly the results reported here. The concentration (cells/milliliter) of the minority parental type in the mating mixture, $N(0)$, as measured by ability to produce colonies when grown on proper selective growth media is determined at the start of the mating. Under the conditions used here the number of recombinants is directly proportional to $N(0)$ and is essentially independent of the concentration of the majority parent. In some experiments the minority parent is subjected before mating to a dose, D, of an injurious agent (e.g., X-rays). The fraction of cells surivivng such a treatment is $(N(D)/N(0)$, where $N(D)$ is the concentration of viable cells after irradiation. At the end of the mating period, aliquots are plated on various selective media which contain streptomycin and are either deficient in one of the amino acids required for growth by the female parent or which contain galactose instead of glucose. Recombinant production is assayed by colony formation after incubation at 37°C for 36 hr. The number of recombinant cells/milliliter inheriting a specific male marker *a* and the female marker *str*^{s} is a function of the positions of those markers on the male chromosome and of the dose, D, of an injurious agent. This will be written as $R(a, \bar{s}; D)$ where a bar over a marker symbol denotes the female allele.

For purposes of a genetic analysis recombinants are selected which have specific genetic characters (selected markers) and are then analyzed for the presence of other characters (unselected markers). Such analyses can be performed under different external conditions (e.g., previous exposure to ionizing radiation). We will write the number of recombinant cells/milliliter inheriting male markers, $a, b, \cdots$ and female markers $r, s, \cdots$ as $R(a, b, \cdots,$

$\bar{r}, \bar{s}, \cdots ; D$). The fraction of these subsequently analyzed for the male markers $c, d, \cdots$ and female markers $t, u, \cdots$ will then be written as

$$G\ (c, d, \cdots \bar{t}, \bar{u} \mid a, b, \cdots \bar{r}, \bar{s}, \cdots ; D)$$

III. NORMAL CONJUGATION

A. *An Analysis of the Steps of Conjugation*

The number of recombinants having a male marker a and the female marker s has been designated as $R(a, \bar{s}; 0)$ and is proportional to $N(0)$, the number of cells of the minority parental class. Thus the probability per minority parent for the formation of this recombinant class if $R(a, \bar{s}; 0)/N(0)$ which we designate as "marker presence." The appearance of recombinant types following mating can be taken as evidence that the successive steps of union, transfer, and integration-segregation have occurred for these cells. We assume that the steps of conjugation are independent and write

$$R(a, \bar{s}; 0)/N(0) = P_u \cdot P_t \cdot P_{is} \qquad (1)$$

where P_u, P_t, and P_{is} are the probabilities of occurrence for the steps of union, transfer, and integration-segregation, respectively. We discuss next these probabilities in greater detail in an attempt to elucidate their functional dependence on male marker position using as a descriptive parameter the position x of the selected marker on the male chromosome.

1. *Probability of Union* (P_u). Union is defined herein to include all those processes which precede the actual transfer of a male marker that is arbitrarily close to the origin of the male chromosome. Thus a successful union would consist of the following successive processes: (1) a collision between a donor cell and a recipient one; (2) an attachment between colliding cells that is sufficiently stable to allow subsequent events to occur; (3) the mobilization of the conjugating system for the initiation of transfer of a male chromosome into the female.

The zygotic induction experiments of Jacob and Wollman (1961) indicate that processes (1) and (2) occur with a probability approaching 1 under proper mating conditions. Experiments to be discussed show that process (3) under our experimental conditions of extended mating times has a high probability of occurrence approaching 1. Therefore, the probability of union, P_u, is close to 1. This probability should be the same for all markers on the chromosome since it involves an interaction between cells; hence P_u is independent of marker position; that is, independent of x.

2. *Probability of Integration-Segregation* (P_{is}). Jacob and Wollman have concluded by use of the technique of "zygotic induction" that all male markers

known to be transferred to the zygote have the same probability of being incorporated into the recombinant chromosome; that is, that there is no gradient of integration (1961). Clark and Adelberg (1962) have questioned this interpretation and have concluded from the data of Jacob and Wollman that there is a progressive change along the chromosome in the probability of integration. An analysis of the unpublished data will reveal that the probable statistical errors were such as to make Jacob and Wollman's interpretation valid.

Experiments to be discussed in detail later (section IIIC-2) strongly confirm the invariance of P_{is} with position. Briefly, if recombinants are selected which have a distal male marker and are analyzed for the presence of unselected male warkers, it is found that these markers have the same frequency of appearance (about 0.5 for the strains used here; see Verhoef and deHaan (1966) for a more detailed discussion). Therefore widely separated markers that are transferred have the same probability of being utilized in the recombinant; that is, there is no measurable gradient in the probability of integration. Furthermore, since a stable recombinant chromosome formed in the zygote will segregate from the residual genetic information of the zygote as an intact unit, we conclude that P_{is} is the same for all markers and independent of x.

3. *Probability of Transfer (P_t) and the Generation of Chromosome Maps.* $R(a, s; 0)$ is strongly dependent on the position of a and decreases with the distance between the origin and the selected marker. From the experimental results and the previous discussion we conclude that P_u and P_{is} are position-independent. We conclude therefore that the positional dependence of a resides completely in P_t.

It is necessary to define in an operational way distances between markers on the male or female chromosome. The four basic methods for obtaining chromosome maps for this system—recombinational analysis, times of entry of male markers, the gradient of marker transmission ("marker presence"), and relative radiation sensitivities of marker presence—have been reviewed by Hayes (1964). The second and third methods are discussed below and the fourth in section IV.

In experiments designed to determine entry times of donor markers, Hfr and F^- cells are mixed together under standard mating conditions; aliquots from this mating mixture are removed at various time intervals and mating is interrupted by violent mechanical agitation (or by lysis from without). It is found that a given male marker appears in the recombinants produced after such treatment only after a specific time (the entry time), and that the various markers can thereby be ordered into a unique sequence for a particular male strain. The minimum entry time determined in this way consists of two components: (1) the minimum time required for union (collision, stabilization, mobilization, etc.); and (2) the minimum time required to transfer the male chromosome segment between the origin and the selected marker. Low (1965) has concluded that the first time interval is about 4 min at 37°C. Furthermore the time interval between two specific markers is the

same for various Hfr strains evn though the distances between the origin and the markers may be greatly different (Hayes, 1957; Taylor and Thoman, 1964). From this fact and from studies on the effects of temperature on entry times (Wood, 1968) it appears likely that the rate with which the male chromosome is transferred into the female is approximately constant under a given set of mating conditions. Thus the differences in transfer times between pairs of markers on the male chromosome would be directly proportional to physical distances between the marker pairs. It is relatively easy to obtain accurate maps with this technique (Taylor and Trotter, 1967; Low and Wood, 1965); in this paper all marker positions have been obtained from such experiments, proper corrections (4 min) having been made for mobilization time.

If matings are not interrupted as in the above type of experiment, but are permitted to continue for a time long enough to allow the transfer of the region of the male chromosome being studied and not long enough to allow recombinant cell division, it is found that different male markers appear in the recombinant population with different frequencies. If the markers are ordered into a sequence corresponding to their frequencies of appearance, this sequence of markers is identical to that generated by the interrupted mating type of experiment. Since only P_t is position-dependent, it is reasonable to assume that random events occurring in the male chromosome prevent the transfer of genetic material distal to the position of the most proximal event (Jacob and Wollman, 1961). Such interruptions might be due to breakage of the DNA being transferred, or to breaks or lesions in the template DNA which would either interrupt synthesis or modify it in such a way that transfer would be halted at the site of the most proximal lesion (Jacob, Brenner, and Cuzin, 1963). In addition, breaks in the DNA in the transfer tube (the pilus) (see Brinton, 1965) might occur. We assume tentatively that there is a constant probability per unit distance along the chromosome for such interruptions. We shall write the constant as ν^0_{mt}, where the subscripts denote the parental chromosome and the process and the superscript indicates the absence of radiation. Therefore the probability of transfer as modified by random breakage can be written as

$$P_t = e^{-\nu^0_{mt} x_a} \tag{2}$$

where x_a is a measure of the distance of the selected marker a from the origin (Jacob and Wollman, 1961). Since marker presence is proportional to P_t, for markers not too close to the origin, we have

$$R(a, \bar{s}; 0)/N(0) = A(0)e^{-\nu^0_{mt} x_a} \tag{3}$$

where $A(0)$ is a position-independent factor. From experimental values of marker presence a chromosome map may be constructed which gives the relative distances between markers. Such a map, when normalized, is identical to that produced by

the interrupted mating technique. This suggests that the assumptions of both techniques are proper (constant velocity of transfer along the chromosome and constant probability of interruption of transfer per unit length) or that variations in either assumption are exactly compensated for by variations in the other, a highly unlikely possibility.

Jacob and Wollman (1961) have found that ν^0_{mt} has an approximate value of 0.06 min^{-1}. A more detailed study of this parameter (Wood, 1968) gives the value 0.064 $\pm$ 0.004 min^{-1} and shows that it is approximately invariant to donor or recipient strain differences, to previous growth conditions, to mating temperature, and to degree of agitation during mating (liquid or membrane filter matings).

B. *Association Functions and Genetic Recombination*

Two steps are assumed to take place in the formation of a recombinant chromosome: (1) the transferred male genetic material and the female chromosome come into at least partial juxtaposition; and (2) the recombinant chromosome is formed by utilizing genetic information from the parental chromosomes. Classically, the recombination process has been considered to be essentially symmetric between the two parental chromosomes. It is upon this assumption that the association functions between markers have normally been calculated (Walmsley, 1969). However, this assumption is not necessary a priori, and when parental bacteria are subjected to radiation, as in the experiments to be discussed later, it is clearly incorrect. Lethal lesions and additional cross-over events are introduced asymmetrically and severely modify the apparent linkage between markers. In the most general case there are four possible association functions. They will be written $L_M{}^m$, $L_M{}^f$, $L_F{}^m$, and $L_F{}^f$, where the subscripts and superscripts denote the parental chromosome contributing the selected and unselected markers, respectively. Thus $L_M{}^f$ is the probability that a recombinant having a given selected male marker will also have a particular unselected female marker. These associated functions have four parameters: ν_{mr} and ν_{fr}, the densities of recombinational sites on the male and female parental chromosomes, respectively; and ν_{ml} and ν_{fl}, the densities of lethal lesions on the two parenteral chromosomes, respectively.

The derivations of the four association functions are given elsewhere (Walmsley, 1969) and the results are summarized in Appendix A. In the special case in which there are no lethal lesions on either parental chromosome $L_M{}^f + L_M{}^m = 1$ and $L_F{}^f + L_F{}^m = 1$ for all values of separation distances, x. In the general case the presence of lethal lesions causes these sums to decrease as x increases.

In the case of symmetric recombination we have $\nu^0_{mr} = \nu^0_{fr} \equiv \nu_r{}^0$. If, in addition, there are no lethal lesions, then the frequency of crossing-over between two markers separated by a distance x is given by (see Appendix A):

$$L_M{}^f(x) = L_F{}^m = \tfrac{1}{2}(1 - e^{-2\nu_r{}^0 x}). \tag{4}$$

For distances sufficiently short that the probability of crossing-over is small ($2\nu_r{}^0x \ll 1$), this becomes approximately

$$L_M{}^f(x) = L_F{}^m(x) = \nu_r{}^0x \tag{5}$$

the familiar result of classical genetics (Haldane, 1919).

C. *Marker Presence and Genetic Analyses*

1. *Marker Presence.* It is well-established from both physical observation (Cairns, 1963) and from genetic linkage (Taylor and Thoman, 1964) that the chromosome configuration of the F^- cell (the female parent) is circular. Recombinant cells, with rare exception, are F^- and should have the circular chromosome configuration. In Fig. 1 the female chromosome is represented by the inner closed circle and the transferred male chromosome (usually incomplete), by the outer arc. Our selective procedure demands that a selected male marker (e.g., *a*) and a female marker (e.g., *s*, usually streptomycin) be utilized. To obtain a closed configuration odd numbers of recombinational events must occur both between the origin and x_a,[1] and between x_a and x_s; an even number must occur between x_s and x_E where x_E is the length of the entire chromosome.

In calculating the probability of occurrence of recombinants having the male marker at x_a and the female marker at x_s, or marker presence $R(a, s; 0)/N(0)$, we must consider the contributions of four classes of zygotes which are determined by x, the length of transferred male chromosome which may vary between 0 and x_E.

Class I ($x < x_a$). This class contributes nothing as the male marker at x_a is not transferred.

Class II ($x_a < x < x_s$). There must be an odd number of recombinational events both between 0 and x_a, and between x and x_s; remembering that the probability that the male chromosome terminates between x and $x + dx$ is $P_t(x)\nu_{mt}^0\,dx$, we may write the contribution of this class as

$$KL_F{}^m(x_a)\int_{x_a}^{x_s} L_M{}^f(x - x_a)P_t(x)\nu_{mt}^0\,dx \tag{6}$$

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[1] There is some evidence that simple association functions of the type considered and used here are not applicable for markers within a few minutes of the origin (Low, 1965). If pairing between the proximal end of the male chromosome and the female one is mandatory, then this region may be considered a region of high negative interference and the effective density of recombinational sites may be higher than average; in regions that are paired the recombinational coefficients have been estimated to be approximately 20 recombinational units/min (Jacob and Wollman, 1961; Low, 1965) while average values of approximately 5 recombinational units/min have been reported (Verhoef and deHaan, 1966; Wu, 1967; section IIID). None of the markers utilized in this study are extreme proximal ones so the association functions have the forms given here.

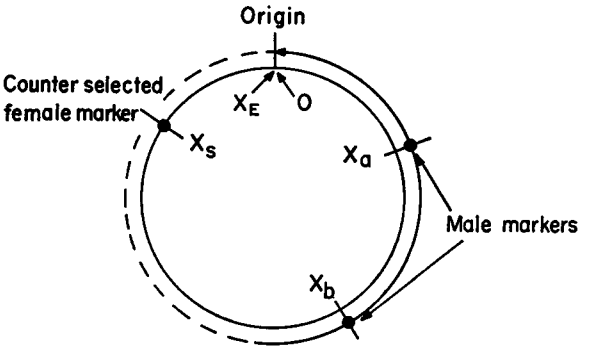

FIGURE 1 Diagram for recombinant analysis. The inner circle represents the female chromosome, the outer one, the male. The dashed portion of the male chromosome is not known to have been transferred.

where K is a constant which includes the probabilities associated with union, initiation of transfer, segregation, and all other processes of recombination which do not depend on the locations of the genetic markers.

Class III ($x_s < x < x_E$). In a similar way we may write the contribution of this class:

$$KL_F{}^m(x_a)L_M{}^f(x_s - x_a)\int_{x_s}^{x_E} L_F{}^f(x - x_s)P_t(x)\nu_{mt}^0\,dx. \tag{7}$$

Class IV ($x = x_E$). In class III it was obligatory for an odd number of recombinational events to occur between the origin and x_a and an even number between x_s and x_E. If the entire male chromosome is present this is not necessary (Fulton, 1965) and the following additional contribution must be added:

$$KL_M{}^f(x_s - x_a)\,L_F{}^m(x_E - x_s + x_a)\,P_t(x_E). \tag{8}$$

Collecting all terms we have

$$\begin{aligned} R(a, s; 0)/N(0) = K\Big\{ & L_F{}^m(x_a)\int_{x_a}^{x_s} L_M{}^f(x - x_a)P_t(x)\nu_m^0\,dx \\ & + L_F{}^m(x_a)L_M{}^f(x_s - x_a)\int_{x_s}^{x_E} L_F{}^f(x - x_s)P_t(x)\nu_{mt}^0\,dx \\ & + L_M{}^f(x_s - x_a)L_F{}^m(x_E - x_s + x_a)P_t(x_E)\Big\}. \end{aligned} \tag{9}$$

Similarly the presence of recombinants having male markers both at x_a and x_b and the female marker at x_s (Fig. 1) is given by

$$R(a, b, \bar{s}; 0)/N(0) = K\Big\{L_F^{m}(x_a)L_M^{m}(x_b - x_a)\int_{x_b}^{x_s} L_M^{f}(x - x_b)P_t(x)\nu_{mt}^0\,dx$$

$$+ L_F^{m}(x_a)L_M^{m}(x_b - x_a)L_M^{f}(x_s - x_b)\int_{x_s}^{x_E} L_F^{f}(x - x_s)P_t(x)\nu_{mt}^0\,dx$$

$$+ L_M^{m}(x_b - x_a)L_M^{f}(x_s - x_b)L_F^{m}(x_E - x_s - x_a)P_t(x_E). \qquad (10)$$

Since all the functions are known, the two integrations can be carried out and are presented in Appendix B.

In the usual case in which markers a and s are well-separated ($e^{-\nu_{mt}^0(x_s-x_a)} \ll 1$), only the first term in equation 9 is important. This is equivalent to saying that the normal gradient is sufficiently steep that one can neglect the contributions of zygotes in which the male chromosome has been transferred as far as the counter-selection marker. This gives

$$R(a, \bar{s}; 0)/N(0) = K\frac{\nu_{fr}^0 \cdot \nu_{mr}^0}{(\nu_{fr}^0 + \nu_{mr}^0)(\nu_{fr}^0 + \nu_{mr}^0 + \nu_{mt}^0)}e^{-\nu_{mt}^0 x_a} = A(0)e^{-\nu_{mt}^0 x_a} \qquad (11)$$

where $A(0)$ is a position-independent constant. This result is the justification for equation (3) in section IIIA-3 in which the proportionality between marker presence and the transfer function $P_t(x)$ was used to construct a genetic map of the chromosome.

2. *Genetic Analyses of Proximal Unselected Markers.* The fraction of those recombinants having a male marker b and a female marker s, which also has an *unselected* male marker a is given by:

$$G(a \mid b, \bar{s}; 0) = \frac{R(a, b, \bar{s}; 0)}{R(b, \bar{s}; 0)}. \qquad (12)$$

Providing that the markers are sufficiently far from the origin and the counter-selection marker that we may neglect $e^{-\nu_{mt}^0(x_s-x_b)}$ and $e^{-(\nu_{fr}^0+\nu_{mr}^0)x_a}$ compared to 1, this expression reduced to

$$G(a \mid b, \bar{s}; 0) = L_M^{m}(x_b - x_a) = \frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0}\left[1 + \frac{\nu_{mr}^0}{\nu_{fr}^0}e^{-(\nu_{mr}^0+\nu_{fr}^0)(x_b-x_a)}\right] \qquad (13)$$

For closely spaced markers this becomes $1 - \nu_{mr}^0(x_b - x_a)$. For well-separated markers one obtains the asymptotic value of $\frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0}$.

In Fig. 2 experimental values for $G(a \mid b, \bar{s}; 0)$ are plotted as a function of the separation between the selected and unselected male markers $(x_b - x_a)$. The asymptotic value of the experimental data is about $\frac{1}{2}$. This implies $\nu_{mr}^0 = \nu_{fr}^0$ as one

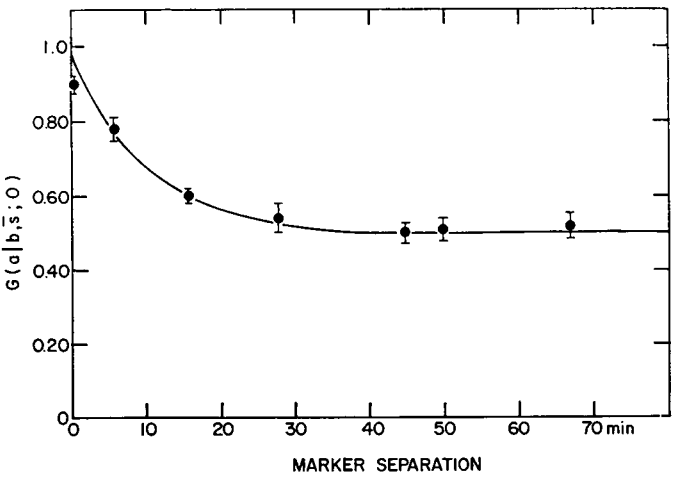

FIGURE 2 Linkage between male markers as a function of marker separation for proximal unselected markers. The solid curve is the best fit to the relationship $L_M{}^m(x) = \frac{1}{2}(1 + e^{-2\nu_r{}^0 x})$ using the value $\nu_r{}^0 = 0.050\ \text{min}^{-1}$. The experimental values are averages obtained from many experiments with several Hfr and F^- strains.

might expect in an isogenic mating. Under manifestly asymmetric conditions, as when the parental DNA strands are from different strains, one might expect an asymptotic value different from $\frac{1}{2}$. Such observations have been reported (Verhoef and deHaan, 1966).

The fact that the experimental values for $L_M{}^m(x)$ do not vary for separation distances greater than 15 min (about $\frac{1}{6}$ of the chromosome length) indicates that all distant proximal markers have the same probability of integration; that is, there is no measurable gradient of integration. This finding strongly supports the earlier findings of Jacob and Wollman (1961) that were discussed previously in section IIIA-2.

3. *Genetic Analysis of Distal Unselected Markers.* The fraction of recombinants having the male marker a and the female marker s which also have the male marker b is given by

$$G(b \mid a, \bar{s}; 0) = \frac{R(a, b, \bar{s}; 0)}{R(a, \bar{s}; 0)}. \tag{14}$$

Using the same approximations as before, this becomes

$$G(b \mid a, \bar{s}; 0) = \frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0}\left[1 + \frac{\nu_{mr}^0}{\nu_{fr}^0} e^{-(\nu_{mr}^0 + \nu_{fr}^0)(x_b - x_a)}\right] e^{-\nu_{mt}^0 (x_b - x_a)}. \tag{15}$$

This result could have been obtained directly since it is the probability that the male chromosome segment between x_a and x_b be transferred multiplied by the linkage

between the two markers. Since $\nu^0_{fr} \approx \nu^0_{mr}$ equation 15 becomes, for unlinked markers

$$G(b \mid a, \bar{s}; 0) = \tfrac{1}{2} e^{-\nu^0_{mt}(x_b - x_a)} \qquad (16)$$

a relationship that agrees well with experiment (Marcovich, 1961; Krisch, 1965; Joset and Wood, 1966).

D. *Parameters of the Analysis and their Invariance*

The experimental results discussed thus far can be analyzed in terms of equations 11, 13, and 16, which involve only the two experimental constants: ν^0_{mt} (the gradient number) and $\nu^0_{fr} \approx \nu^0_{mr} = \nu_r{}^0$. The slope from the gradient plot ($R(a, \bar{s}; 0)/N(0)$ against x_a) gives a direct measure of ν^0_{mt}. The average value of this parameter (Wood, 1968) is 0.064 ± 0.004 min^{-1}. This is equivalent to the statement that on the average there are approximately six sites on the male chromosome which produce operational discontinuities during transfer, or that the probability of interruption per nucleotide pair during transfer is about 10^{-6}.

The constant associated with the integration process, $\nu_r{}^0$, can be evaluated by obtaining the best fit to the data of Fig. 2 using equation 13. We obtain $\nu_r{}^0 \approx 0.05 \pm 0.01$ min^{-1}; on the average there are about five recombination events that occur during integration over the entire length of the chromosome. Thus the probability of such an event per nucleotide pair is about 10^{-6}. Similar estimates for this parameter have recently been reported by others (Verhoef and deHaan, 1966; Wu, 1967).

The data in Fig. 2 indicate that 1 min of marker separation is equivalent to five recombinational units. Jacob and Wollman (1961) have found that for markers separated by 2–3 min, 1 min of marker separation is equivalent to 20 recombinational units. Recombination between very close markers would involve regions of negative interference while recombination between distant markers would involve regions of both negative and positive interference. Thus the relation between distance and recombinational units found in the present study represents an average over large chromosomal regions.

This rather striking correspondence in the values of ν^0_{mt} and $\nu_r{}^0$ is interesting since it can be interpreted to mean that the causes of transfer "interruptions" and recombination "events" may have a common root. This speculation will be examined further in later sections.

IV. IRRADIATION OF THE DONOR PARENT

The fact that the several steps in the conjugation process may be separated analytically makes possible an understanding of studies in which injurious agents are applied to either the male or the female that affect selectively the sequential steps (Joset and Wood, 1966). It has been possible through such studies to obtain a better understanding of both conjugation and the mode of action of injurious agents.

Fuerst, Jacob, and Wollman (1956) found that the radioactive decay of ^{32}P incorporated into the genome of HfrH selectively prevented appearance of donor markers in recombinants. The decay sensitivities for the loss of appearance of the various male markers were found to be directly proportional to the distances of the markers from the origin as determined by interrupted mating experiments. Marcovich, using markers distributed over a larger region of the male chromosome, has studied the effect of X-rays applied to the donor parent prior to mating (1961). His results were similar to those above and indicate that such studies may be used to generate chromosome maps.

^{32}P decay, X-rays, α-particles, and ultraviolet light are found experimentally to have quite similar effects on conjugation. In the following section a complete analysis will be made of the effects on conjugation of α-particle irradiation of the donor prior to mating. Other studies using other radiations will be reviewed in subsequent sections.

A. *α-Particle Irradiation*

1. *Effect on Transfer.* If male cells are exposed to various doses, D_m, of ionizing radiations before mating, it is reasonable to assume that radiation lesions are induced at random along the male chromosome which may operationally produce additional interruptions during the transfer step of conjugation. A new breakage coefficient, ν_{mt}, should now be used in the transfer function which should increase linearly with dose (Marcovich, 1961):

$$\nu_{mt} = \nu^0_{mt} + \sigma_{mt} D_m \tag{17}$$

where σ_{mt} is the average probability per unit length of the chromosome per unit dose for the production of additional transfer lesions. (Actually, the primary radiation effect should be proportional to the electron density; the resolution of the methods used here is such that we can measure only an average effect that is over a fairly extended region of the chromosome, say about 10^5 nucleotides).

2. *Effect on Union, Integration, and Segregation.* We may rewrite equation 11 as

$$R(a, \bar{s}; D_m)/N(0) = A(D_m) e^{-(\nu^0_{mt} + \sigma_{mt} D_m) x_a} \tag{18}$$

where we have assumed that $A(P_u \cdot P_{is})$ is also dose-dependent; i.e., union and integration-segregation may be affected by irradiation. $R(a, \bar{s}; D_m/N(0)$ as a function of x_a (the gradient plot) is shown in Fig. 3 for various doses of α-particle irradiation delivered to the donor cells before mating. The extrapolates of the gradients for various doses can be seen to intersect at a common point. Equation 18 predicts that the value of $R(a, \bar{s}; D_m)/N(0)$ for $x_a = 0$ should be $A(D_m)$. Since the curves have a

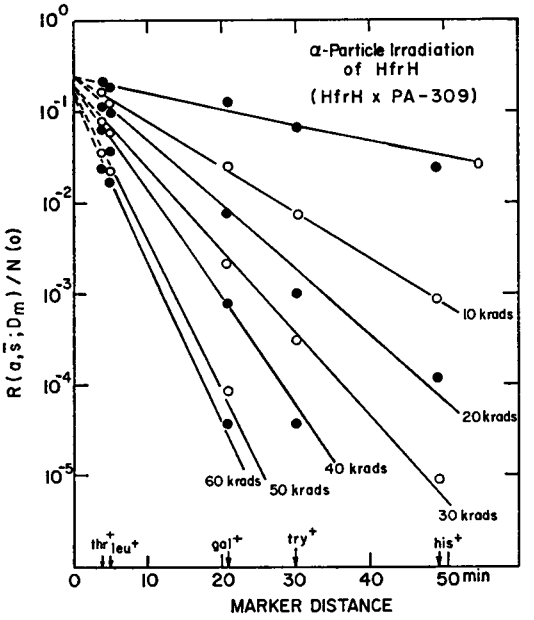

FIGURE 3 Effect of α-particle irradiation of the donor parent on the gradient plot.

common intersection, A is dose-independent; that is, it has the value $A(0)$. This means that the α-particle irradiation has no over-all effect on the processes of union and integration-segregation. Thus male cells that are unable to produce colonies after irradiation may, nevertheless, mate.

We may write, using equation 18 and the fact that $A(D) = A(0)$

$$R(a, \bar{s}; D_m)/R(a, \bar{s}; 0) = e^{-\sigma_{mt} x_a D_m}. \quad (19)$$

Experimental values of this function are plotted for various values of x_a in Fig. 4 as a function of α-particle dose D_m. To within the experimental errors these curves are exponential as predicted by equation 19, and the experimental slopes from these marker survival curves should be proportional to the distance between the origin and the selected marker. In Fig. 5 these slopes are plotted against the distance of the various markers from the origin as determined by interrupted mating experiments, with proper corrections made for mobilization time. A linear relationship exists within the experimental errors. This approach can thus be used to produce a type of chromosome map in which the distances are related to over-all marker radiosensitivities since the radiosensitivity per unit length would be expected to be approximately constant over the distances that can be resolved here. This method provides a third independent way to construct a chromosome map (see section IIIA-3 and Joset and Wood [1966]).

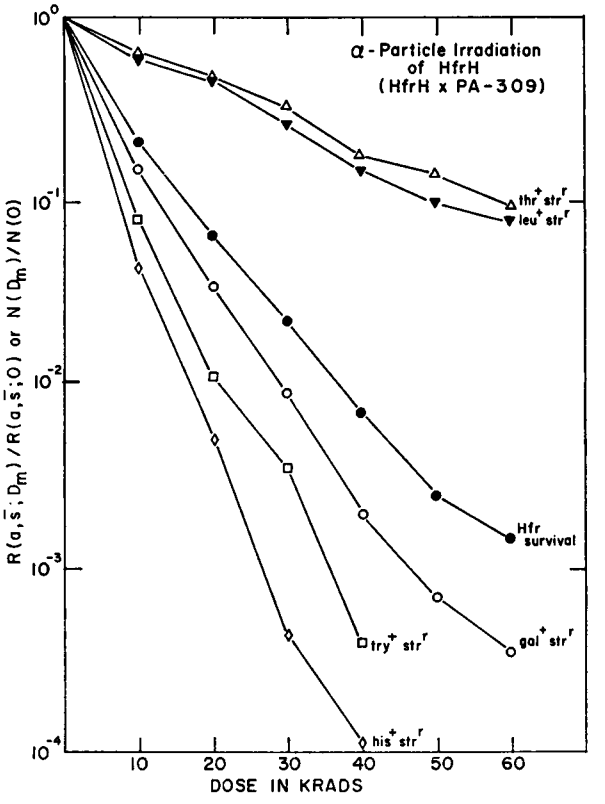

FIGURE 4 Effect of α-particle irradiation of the donor parent on marker survival, $R(a,\bar{s}; D_m)/R(a,\bar{s}; 0)$.

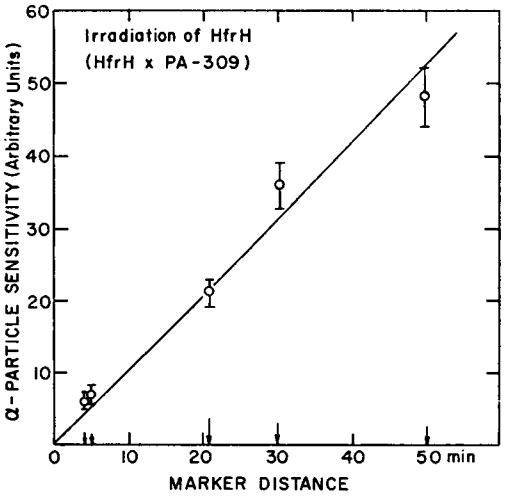

FIGURE 5 The relationship between the slopes of the marker survival curves (Fig. 4) and marker position.

3. *Effect on Genetic Analysis.* The genetic analysis of the above type of experiment, involving α-irradiation of the donor, is shown in Fig. 6. These results can be separated into two classes: Class I, unselected proximal markers $G(a \mid b, \bar{s}; D_m)$; and Class II, unselected distal markers $G(b \mid a, \bar{s}; D_m)$.

Class I. It can be seen that the frequency of appearance of an unselected proximal marker is not affected by dose; that is, the linkage does not change with dose (e.g., the appearance of all other markers when the selected marker is histidine). It is

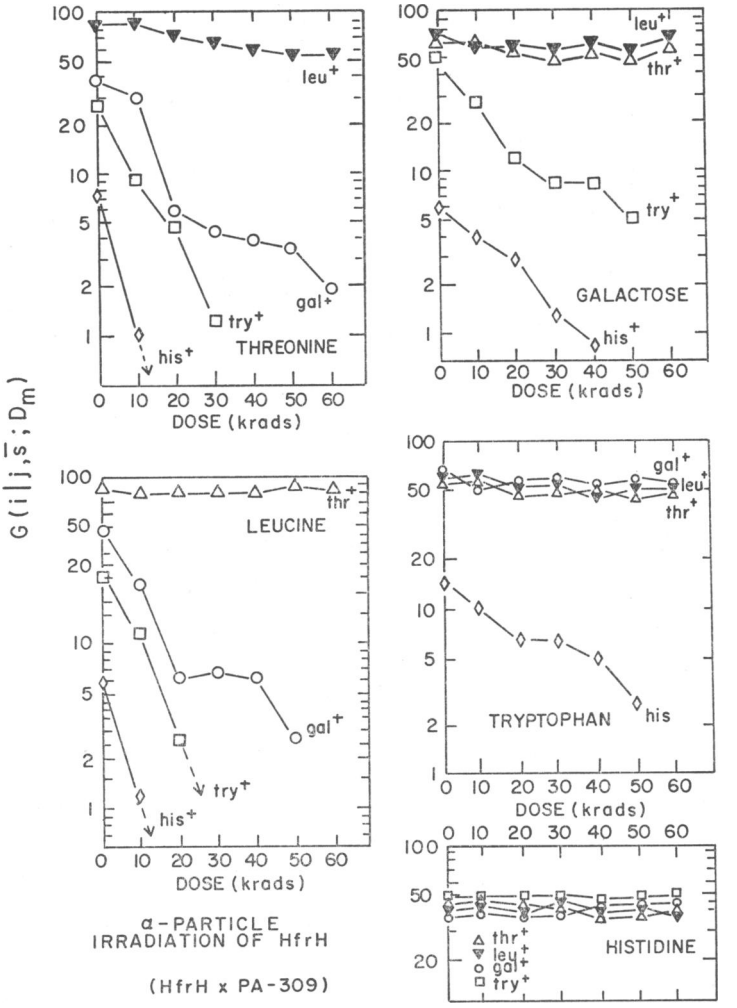

FIGURE 6 Effect of α-particle irradiation of the donor parent on the presence of unselected markers in a population of recombinants selected for a particular donor character.

reasonable to assume that some fraction of the radiation lesions produced on the male chromosome could be transferred to the zygote. During the subsequent steps of integration these lesions could disturb the normal genetic analysis. For example, linkage between male markers is significantly decreased by irradiation of the zygote (Wann and Wood, 1967). The fact that the linkage relationships are dose-independent for proximal unselected markers suggests that radiation lesions on the male chromosome that could serve as additional recombinational events are not transferred into the zygote. If the transfer process serves operationally to remove radiation lesions from the male chromosome, it is reasonable to assume that it has the same role in removing spurious or random lesions in the nonirradiated case. The male chromosome that appears in the zygote, therefore, should effectively have been "cleared" by the transfer process. The numerical equivalence of ν_{mt}^0 and ν_r^0 led to the speculation in section IIID that they arose from a common source. If the male chromosome is cleared of transfer breaks, and hence of recombinational lesions (if they are identical), there would be an asymmetry with respect to such lesions in the female chromosome and the transferred male chromosome. However, it is reasonable to consider that both "transfer" interruptions and integration events that occur with no irradiation arise spontaneously and may be considered to be related to the equilibrium "noise" of the system. Thus introduction of the donor chromosome into the recipient cell would reestablish the normal density of recombinational events.

Class II. Since the linkage function (equation 15) is dose-independent, the only dependence of $G(b \mid a, \bar{s}; D_m)$ on dose is through the transfer function. Therefore, using equation 17, we rewrite equation 15 as

$$G(b \mid a, \bar{s}; D_m) = \tfrac{1}{2}[1 + e^{-2\nu_r^0(x_b - x_a)}]e^{-(\nu_{mt}^0 + \sigma_{mt} D_m)(x_b - x_a)} \qquad (20)$$

where we have used the experimental facts that $\nu_{mr}^0 \approx \nu_{fr}^0 \equiv \nu_r^0$. Using equation 18, we may rewrite equation 20 in the form

$$G(b \mid a, \bar{s}; D_m) = L_M{}^m(x_b - x_a) \frac{R(b, \bar{s}; D_m)}{R(a, \bar{s}; D_m)} . \qquad (21)$$

The values of the functions on the right side of equation 21 can be obtained for different values of D_m from the data of Figs. 4 and 6. Values of $G(b \mid a, \bar{s}; D_m)$ computed in this way are in good agreement with the experimental values.

B. *X-Ray Irradiation*

Completely comparable experimental results on this same system have previously been obtained by Marcovich using X-irradiation (1961). We have verified Marcovich's results and the system constants for X-rays are summarized in Table I.

TABLE I

COEFFICIENTS FOR THE PRODUCTION OF CHROMOSOMAL LESIONS BY RADIATIONS

Type lesion	X-rays	α-Particles	^{32}P decay
	$min^{-1}\ krad^{-1}$	$min^{-1}\ krad^{-1}$	$min^{-1}\ (mc/mg)^{-1}$
Transfer (σ_{mt})	0.003	0.006	0.005
Recombinational (σ_r)	0.004	0.008	0.007

σ_{mt} is computed for α-particles from the data of Fig. 4; for X-rays, from Marcovich (1961); for ^{32}P, from Krisch (1965).

σ_r is computed from the genetic analysis of proximal, unselected markers (X-rays and α-particles: Wood and Marcovich, 1964; ^{32}P: Krisch and Wood, 1965) and equation 28. For distant markers, $G(a \mid b;\bar{s};D_f) = \frac{1}{2}(1 + \delta)$; from the definition of δ (Appendix A) and the experimental result $\nu_{fr}^0 = \nu_{mr}^0 = \nu_r^0$, we find $\frac{dG}{dD} = \frac{\sigma_r + \sigma_{fl}}{\nu_r^0}$ for small values of D. σ_{fl} and ν_r^0 are known and $\frac{dG}{dD}$ can be estimated from plots such as Fig. 9.

C. *Decay of ^{32}P Incorporated into Donor Parent DNA*

Fuerst et al. (1956) have studied the modification of genetic recombination in the K-12 system caused by the decay of radioactive phosphorus incorporated in the male genome. These earlier studies have been extended by Krisch (1965) using additional male markers that allow at least half of the male chromosome to be studied. All the results that were obtained for α-particle irradiation were duplicated with this agent and chromosome maps can be produced that are quantitatively identical, with normalization, to those obtained by the three methods previously discussed (sections IIIA-3 and IVA-2) if the following two assumptions are made: (1) there is a constant probability for incorporation of radioactive phosphorus along the chromosome; and (2) there is a constant probability of breakage lesions being produced per decay in all regions of the chromosome. Both these assumptions are reasonable, at least at the level of resolution that this technique affords (about 10^5 nucleotide pairs).

D. *Ultraviolet Irradiation*

Joset and Wood (1966) have found that the action of ultraviolet irradiation (2537 A) on genetic recombination in this system is more conplicated than that of ionizing radiations. In addition to effects on transfer, ultraviolet irradiation affects the other steps of union and integration-segregation; that is, $A(P_u \cdot P_{is})$ is dose-dependent and has the form $A(D_m) = A(0)e^{-KD_m}$. The fact that the steps may be separated analytically allows a determination to be made on the dependence of the transfer function, P_t, on dose. Marker survival curves similar to those of Fig. 4 may be determined and it is found that their slopes are proportional to marker position as deter-

mined by other techniques. Thus, again, a chromosome map may be determined which, with normalization, is identical to those produced in other ways.

V. IRRADIATION OF THE RECIPIENT PARENT

A. *Summary of Experimental Results*

Wood and Marcovich (1964), Krisch and Wood (1965), and Joset and Wood (1966) have studied the effects on genetic recombination of irradiation of the recipient parent. The radiations used were X-rays, α-particles, ^{32}P decay, and ultraviolet light. The general characteristics of the results were the same in each case and are summarized in Figs. 7–9.

Fig. 7 is the gradient plot showing the dependence of marker presence on distance from the origin at various radiation doses. The distinctive feature is that although the presence of a given marker decreases with dose, the gradient number (ν_{mt}) also decreases. This is to be contrasted with the situation for irradiation of the donor where the gradient increases with dose (Fig. 3).

In Fig. 8 marker survival as a function of dose is shown. Here again the results differ completely from the case of donor irradiation (Fig. 4). In this case the distal markers are *less* sensitive to radiation than proximal markers.

Finally, Fig. 9 shows the dose-dependence results of the "genetic analysis." It can

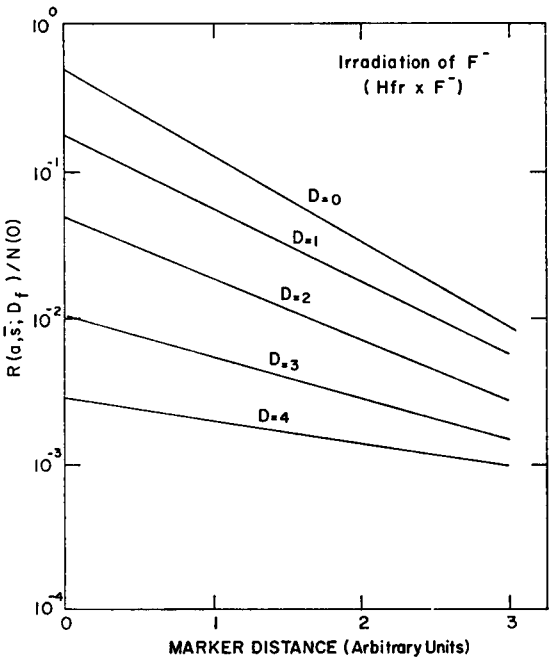

FIGURE 7 Effect of irradiation of the recipient parent on the gradient plot.

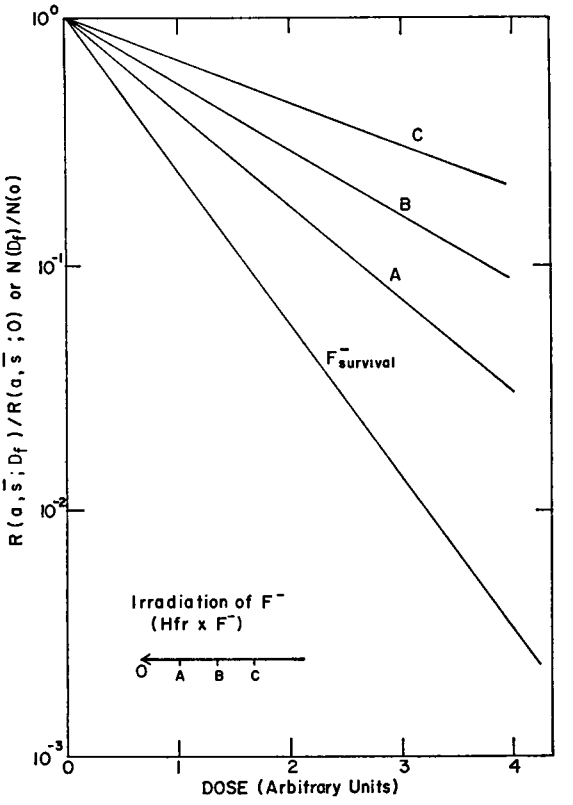

FIGURE 8 Effect of irradiation of the recipient parent on marker survival.

be seen that markers proximal to the selected marker rise slowly toward some asymptotic value, while distal markers rise rapidly to values comparable to those of proximal markers. Whether the asymptotic value is unity or less than unity is not yet clear experimentally.

It will be our purpose in this section to show that these rather complicated general results can be accounted for in a very simple and natural way. Whatever the general effects of radiation on the mating system are, the only ones which should affect the genetic properties of the recombinants are those which directly affect the chromosomal recombination process. We will assume that the modifications of genetic recombination by radiation will be due to an increase in the density of recombinational sites and the production of lethal lesions on the female chromosome. One can see qualitatively that the survival of distal markers should be less sensitive to radiation than that of proximal markers: recombinants containing distal markers will contain fewer lethal lesions in the female portion of the chromosome beyond the terminus of the male fragment and will have a longer proximal distance in which to incorporate the male information. Selective utilization of nonirradiated donor ma-

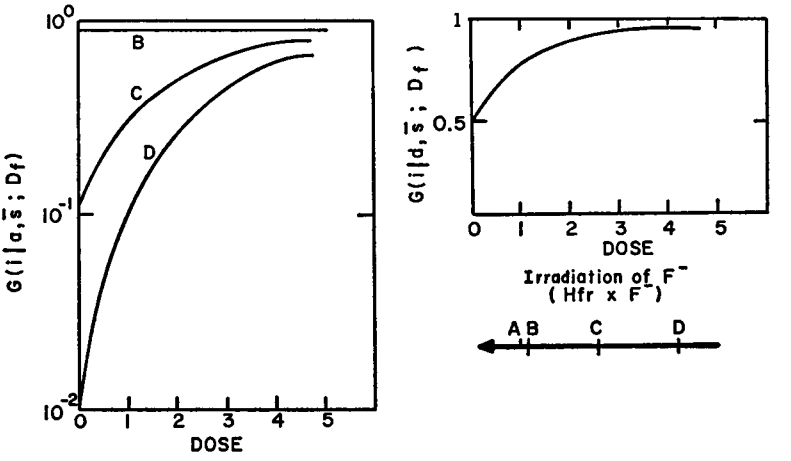

FIGURE 9 Effect of irradiation of the recipient parent on the presence of unselected markers in a population of recombinants selected for a particular donor character. Dosage indicated by arbitrary units.

terial will produce an increased linkage between male markers with dose. These results will now be formulated in a quantitative way.

B. *Marker Presence*

In writing down the expression for marker presence, we may proceed as before with two additional considerations to be kept in mind. First, the association functions themselves are now functions of dose. Second, each contribution to the sum must be multiplied by the probability that there should be no lethal lesions in the remainder of the female chromosome beyond the terminus of the male fragment. If the average density of lethal lesions is ν_{fl}, then this probability is given by $P_l(x) = e^{-\nu_{fl}x}$. The marker presence relationships (equations 9 and 10) become:

$$R(a, \bar{s}; D_f)/N(0) = K\Big\{L_F{}^m(x_a)\int_{x_a}^{x_s} L_M{}^f(x - x_a)P_l(x_E - x)P_t(x)\nu_{mt}^0\,dx + L_F{}^m(x_a)L_M{}^f(x_s - x_a)\int_{x_s}^{x_E} L_F{}^f(x - x_s)P_l(x_E - x) \cdot P_t(x)\nu_{mt}^0\,dx + L_M{}^f(x_s - x_a)L_F{}^m(x_E - x_s + x_a)P_t(x_E)\Big\} \quad (22)$$

$$R(a, b, \bar{s}, D_f)/N(0) = K\Big\{L_F{}^m(x_a)L_M{}^m(x_b - x_a)\int_{x_b}^{x_s} L_M{}^f(x - x_b) \cdot P_l(x_E - x)P_t(x)\nu_{mt}^0\,dx$$

$$+ L_F^m(x_a)L_M^m(x_b - {}_a)L_M^f(x_s - x_b) \int_{x_s}^{x_E} L_F^f(x - x_s)P_l(x_E - x) \cdot P_t(x)\nu_{mt}^0 \, dx$$

$$+ L_M^m(x_b - x_a)L_M^f(x_s - x_b(L_F^m(x_E - x_s + x_a)P_t(x_E)\Big\}. \quad (23)$$

The integrated results are given in Appendix B. Note that equations 22 and 23 are identical to equations 9 and 10 except for the inclusion of $P_l(x_E - x) = e^{-\nu_{fl}(x_E - x)}$ in the integrands. Providing that the selected and counterselected markers are sufficiently far apart, we have at low dose $e^{-(\nu_{mt}^0 + \alpha - \nu_{fl})(x_s - x_a)} \ll 1$. In this region only the first integral in the bracket of equation 22 need be retained. On the other hand, at very high dose (female survival $< 10^{-4}$), we have $\nu_{mt}^0 + \alpha - \nu_{fl} < 0$ and the principal contribution is from those recombinants in which the entire male chromosome has been transferred, i.e., the last term in the brackets of equations 22 and 23.

Low dose approximation. Neglecting $e^{-(\nu_{mt}^0 + \alpha - \nu_{fl})(x_s - x_a)}$ and $e^{-(\beta - \alpha)x_a}$ compared to unity, the expression for marker presence (Appendix B) reduces to

$$R(a, \bar{s}; D_f)/N(0) = K\left(\frac{\gamma}{2}\right)^2 \frac{(\nu_{fr}\nu_{mr}\nu_{mt}^0(\beta - \alpha)}{(\nu_{mt}^0 + \alpha - \nu_{fl})(\nu_{mt}^0 + \beta - \nu_{fl})} \cdot e^{-\nu_{fl}x_E} e^{-(\nu_{mt}^0 + \alpha - \nu_{fl})x_a}$$

$$= A(D_f)\, e^{-\nu_{fl}x_E} e^{-(\nu_{mt}^0 + \alpha - \nu_{fl})x_a} \quad (24)$$

where $A(D)$ is independent of marker position and only very weakly (i.e., algebraically) dependent upon dose.

Equation 24 gives a linear decrease of the logarithm of marker presence with marker position with a slope given by $\nu_{mt}^0 + \alpha - \nu_{fl} \approx \nu_{mt}^0 - \frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0}\sigma_{fl}D_f$. At zero dose the negative slope is just ν_{mt}^0, the normal gradient, and it becomes less negative with increasing dose. This agrees with the general experimental results shown in Fig. 8.

If $R(a, \bar{s}; D_f)$ is considered explicitly as a function of dose for a given marker, we have, using the relations $\nu_{fl} = \sigma_{fl}D_f$ and $\alpha - \nu_{fl} \approx -\frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0}\sigma_{fl}D_f$,

$$R(a, \bar{s}; D_f)/N(0) = A(D_f)\, e^{-\nu_{mt}^0 x_a} \exp\left[-\sigma_{fl}\, D_f \left(x_E - \frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0} x_a\right)\right]. \quad (25)$$

Since the dose dependence of $A(D_f)$ is small, equation 25 gives a very nearly linear decrease of the logarithm of marker presence with dose. The negative slope

is seen to be $\sigma_{fl}x_B$ for a marker at the origin ($x_a = 0$) which is the same as that for the survival of the female cells, in agreement with the experimental results in Fig. 8. For $x_B = 90$ min and from the slope of the female survival curve, σ_{fl} is computed to be 0.0008 krad^{-1} min^{-1} for X-rays and 0.002 krad^{-1} min^{-1} for α-particles. An independent and more significant experimental estimate of σ_{fl} can be obtained from Fig. 7, the gradient plot. If the extrapolates ($x_A = 0$) of the independent gradient curves are plotted as a function of dose, a survival curve for the recipient population that has inherited a male marker at the origin is obtained; the probability of formation of such a recombinant is given by equation 22 for $x_A = 0$ and is $Ke^{-\sigma_{fl}x_B D}$. This extrapolate survival curve is indistinguishable from the female survival curve itself and thus has the values given above. Markers at various positions, x_a, are predicted by equation 25 to have slopes which decrease in proportion to their distance from the origin. This is again in agreement with experiment.

High dose approximation. At very large doses we have $\nu^0_{mt} + \alpha - \nu_{fl} < 0$, and the surviving population is dominated by zygotes in which the entire male chromosome has been transferred. From the value of the normal transfer gradient, we find that this occurs for approximately one mating pair in 10^3, so that this approximation holds for dose values sufficiently large that female survival is less than approximately one in 10^4. The radiation studies of Wood and Marcovich (1964), Krisch and Wood (1965), and Joset and Wood (1966) for technical reasons just approached these values of dose. Therefore this region cannot properly be said to have been investigated experimentally. Our theoretical results in this approximation will give limits against which the asymptotic behavior of the experimental data can be compared.

Retaining only the last term in the bracket, equation 22 reduces to

$$R(a, \bar{s}; D_f)/N(0) = B(D_f)e^{-(\nu^0_{mt}+\alpha)x_B} (\alpha \ll \beta) \qquad (26)$$

where $B(D_f)$ is independent of marker position and only weakly dependent upon dose. Since this expression is independent of x_a, the gradient of marker presence is expected to approach zero at high dose, a result experimentally found (Fig. 7).

C. *Genetic Analysis*

1. *Proximal Unselected Markers.* The fraction of recombinants having the male marker at x_b which also have the male marker at x_a is given by

$$G(a \mid b, \bar{s}; D_f) = \frac{R(a, b, \bar{s}; D_f)}{R(b, \bar{s}; D_f)}. \qquad (27)$$

Low dose approximation. When only the first term in the bracket of each of equations 33 in Appendix B is retained and $e^{-(\beta-\alpha)x_a}|$ compared to unity is neglected,

equation 27 reduces to

$$G(a \mid b, s; D_f) = \frac{1}{2}(1 + \delta)\left[1 + \frac{1 - \delta}{1 + \delta} e^{-(\beta-\alpha)(x_b - x_a)}\right]. \quad (28)$$

At zero dose equation 28 reduces to equation 13.

High dose approximation. When only the last term in the bracket of each of equations 33 in Appendix B is retained and $e^{-(\beta-\alpha)(x_B - x_s + x_a)}$ compared to unity is neglected, we obtain equation 28 again. At high dose the exponential term goes to zero so that $G(a \mid b, s; D_f)$ approaches $\frac{1}{2}(1 + \delta)$. The experimental data of Fig. 9 are for two markers sufficiently separated that the exponential term can always be ignored. The smooth monotonic increase toward the value unity agrees with the theoretical dependence of δ upon dose.

2. *Distal Unselected Markers.* For an unselected marker at x_b which is proximal to the counterselection marker at x_s and distal to the selected marker at x_a, we have

$$G(b \mid a, s; D_f) = \frac{R(a, b, s; D_f)}{R(a, s; D_f)}. \quad (29)$$

Low dose approximation. With the same approximations as before, at low dose equation 29 becomes

$$G(b \mid a, s; D_f) = \frac{1}{2}(1 + \delta)\, e^{-(\nu^0_{mt} + \alpha - \nu_{fl})(x_b - x_a)}\left[1 + \frac{1 - \delta}{1 + \delta} e^{-(\beta-\alpha)(x_b - x_a)}\right]. \quad (30)$$

At zero dose this reduces to the normal linkage multiplied by the transfer gradient, as expected. For small dose we have $\alpha - \nu_{fl} \approx -\frac{\nu^0_{fr}}{\nu^0_{fr} + \nu^0_{mr}} \sigma_{fl} D_f$ so that $G(b \mid a, s; D_f)$ *increases* exponentially fast with increasing dose as is observed experimentally (Fig. 9).

High dose approximation. At high dose one obtains the same result as was previously obtained for proximal unselected markers at high dose. This is to be expected since when the entire male chromosome is present there is a complete symmetry between proximal and distal markers.

The experimental results (Fig. 9) show a rapid initial rise of distal markers to levels comparable to those of proximal markers, followed by a slow increase at higher doses. The actual convergence of distal and proximal markers has not been established experimentally since the data do not yet go to the high dose region. Nevertheless, the theoretical results exhibit all the features of the data in a satisfactory way over the regions studied.

VI. SUMMARY AND DISCUSSION

Chromosome maps for *E. coli* Hfr stains can be obtained from a variety of experiments involving marker entry times, recombinational analyses, frequency of appearance of recombinant types after mating, and radiation sensitivities for the transfer of male markers. All these techniques order markers in an unambiguous wav and give proportional separation of markers.

In crosses with Hfr donor strains the frequency of appearance of the recombinant class inheriting a male marker located at a distance x_a from the origin and a female allele located at a position $x_s (x_s > x_a)$ is given bv the marker presence:

$$R(a, \bar{s}; 0)/N(0) = A(0)e^{-\nu^0_{mt} x_a}.$$

$A(0)$ is the product of the probabilities of union and integration-segregation. $A(0)$ can easily be modified by environmental conditions that hinder or prevent pair formation.

On the other hand, the gradient number, ν^0_{mt}, is invariant to many variations in mating conditions and to donor strains; the best estimate for its value is 0.064 ± 0.004 min^{-1}.

Recombinant clones can be assayed for the presence of an unselected male marker (the genetic analysis). If the unselected marker at x_a is proximal to the selected marker at x_b the probability of its appearance, for the case of symmetric recombination, is given by

$$G(a \mid b, \bar{s}; 0) = \tfrac{1}{2}[1 + e^{-2\nu_r{}^0(x_a - x_b)}]$$

where $\nu_r{}^0$, an experimental constant having the value 0.05 ± 0.01 min^{-1}, is the density of recombinational events on the parental chromosomes. For markers which are separated by distances of at least 15 min, G is approximately 0.5. If the unselected marker is *distal* to the selected marker, the probability of its appearance is approximately

$$G(b \mid a, \bar{s}; 0) = \tfrac{1}{2}[1 + e^{-2\nu_r{}^0(x_b - x_a)}]e^{-\nu^0_{mt}(x_b - x_a)}$$

If, prior to mating, the donor parent is treated with ionizing radiations (X-rays, α-particles, ^{32}P decay), recombinant production is diminished. The observed effects are consistent with the idea that these radiations induce lesions in the donor chromosome proportional to dose, D_m, that result in interruption of the chromosome during transfer; no radiation damage appears to be transmitted into the zygote. A proper description of the experimental observation is obtained from the preceding relation-

ships if the gradient number has a component which increases with dose; i.e., $\nu_{mt}(D_m) = \nu^0_{mt} + \sigma_{mt}D_m$. Therefore, for symmetric recombination we have:

$$R(a, \bar{s}; D_m)/N(0) = A(0)e^{-(\nu^0_{mt}+\sigma_{mt}D_m)x_a}$$

$$G(a \mid b, \bar{s}; D_m) = \tfrac{1}{2}[1 + e^{-2\nu_r{}^0(x_b-x_a)}]$$

$$G(b \mid a, \bar{s}; D_m) = \tfrac{1}{2}[1 + e^{-2\nu_r{}^0(x_b-x_a)}]e^{-(\nu^0_{mt}+\sigma_{mt}D_m)(x_b-x_a)}$$

For ionizing radiations $A(0)$ is dose-independent; for ultraviolet irradiation $A(D) = A(0)e^{-KD_m}$.

It is possible to use irradiation of the donor parent as a means of mapping the male chromosome. If experimental values of the ratio

$$R(a, \bar{s}; D_m)/R(a, \bar{s}; 0) = e^{-\sigma_{mt}x_aD_m}$$

are plotted against D_m, the marker survival curve has a slope which is $\sigma_{mt}x_a$; since σ_{mt} should be constant along the chromosome the radiosensitivities ($\sigma_{mt}x_a$) are proportional to marker positions.

However, if recipient cells are irradiated before mating, the transfer process per se is not found to be measurably affected and the changes in the relative frequencies of appearance of the various recombinant types are due to modifications in those steps occurring subsequent to transfer, namely, integration. Radiations would produce lesions in the female chromosome which could enhance recombinational events during integration and which could be lethal if incorporated into the recombinant chromosome. The analysis of these effects using these concepts is much more complicated than the case of male irradiation but is completely consistent with the experimental results (Figs. 7–9). Concisely, there is a preferential utilization of male genetic information in crosses made with irradiated female cells.

Two constants of this conjugation system, the gradient number, ν^0_{mt} (the probability of interruption of transfer per unit distance), and $\nu_r{}^0$ (the probability per unit distance of "switching" or "crossing-over" during integration), are invariant to many physical variations and to male strains. Furthermore, within the experimental uncertainty, these two parameters have the same value, 0.06 min^{-1}.

In these formulations the reasonable assumption is made that both ν_{mt} and ν_r increase linearly with dose, i.e. $\nu_{mt}(D) = \nu^0_{mt} + \sigma_{mt}D$ and $\nu_r(D) = \nu_r{}^0 + \sigma_rD$, where σ_{mt} and σ_r are the additional transfer and recombination lesions induced per unit chromosomal length per unit dose. The best estimates for these X-ray, α-particles, and ^{32}P decay are given in Table I. It is noteworthy that with all three radiations, σ_{mt} and σ_r for a particular radiation have the same values within experimental errors (25%). This considerably reinforces our speculation of section IIID that σ_{mt} and σ_r although arising from two completely different steps of conjugation have a com-

mon origin. At the molecular level this is quite reasonable; lesions in the chromosome, whether induced by natural noise or by radiation, would interrupt the chromosomal continuity. From these results these discontinuities would have equal probabilities of interrupting either the transfer process or the integration process.

Because of the experimental observation that $\nu_{mt}^{0} \approx \nu_{r}^{0}$ a single parameter can be used to describe quantitatively marker presence and the genetic analysis in the absence of radiation. Although irradiation of the donor or recipient parents prior to mating significantly affects in different ways both marker presence and the genetic analysis, the quantitative behavior of the system can be described by the addition of a single additional parameter σ because of the experimental observation that $\sigma_{mt} \approx \sigma_{r}$. These unexpected quantitative results suggest that all these effects are mediated at the chromosomal level and are due to a single type of molecular event.

APPENDIX A

GENETIC RECOMBINATION ASSOCIATION FUNCTIONS

The derivations of the association functions have been given by Walmsley (1969). The functions familiar to the classical geneticist are special cases of these. The generalizations involved are: (1) The probabilities for incorporation into the recombinant chromosome of genetic information from the donor and from the recipient may be different. (2) Either parental chromosome may contain lethal lesions induced by radiation or other injurious agents.

The functions are (the subscripts and superscripts denote the selected and unselected alleles, respectively):

$$L_{M}^{m} = \tfrac{1}{2}(1 + \delta)e^{-\alpha x} + \tfrac{1}{2}(1 - \delta)e^{-\beta x}$$

$$L_{M}^{f} = \tfrac{1}{2}\nu_{mr}\gamma(e^{-\alpha x} - e^{-\beta x})$$

$$L_{F}^{f} = \tfrac{1}{2}(1 - \delta)e^{-\alpha x} + \tfrac{1}{2}(1 + \delta)e^{-\beta x}$$

$$L_{F}^{m} = \tfrac{1}{2}\nu_{fr}\gamma(e^{-\alpha x} - e^{-\beta x})$$

where

$$\alpha = \frac{b}{2} - \sqrt{\left(\frac{b}{2}\right)^{2} - c}$$

$$\beta = \frac{b}{2} + \sqrt{\left(\frac{b}{2}\right)^{2} - c}$$

$$\gamma = \frac{1}{\sqrt{\left(\frac{b}{2}\right)^{2} - c}}$$

$$\delta = \frac{\nu_{fr} - \nu_{mr} + \nu_{fl} - \nu_{ml}}{2\sqrt{\left(\frac{b}{2}\right)^{2} - c}}$$

$$b = \nu_{fr} + \nu_{mr} + \nu_{fl} + \nu_{ml}$$

$$c = \nu_{fr}\nu_{ml} + \nu_{fl}\nu_{mr} + \nu_{fl}\nu_{ml}$$

ν_{fr} is the density of transitions from female genetic information to male genetic information.

ν_{mr} is the density of transitions from male genetic information to female genetic information.

ν_{fl} is the density of female lethal lesions.

ν_{ml} is the density of male lethal lesions.

x is the distance between the two genetic markers.

For the important special case in which there are no lethal lesions, the linkage functions simplify to

$$L_m{}^m = \frac{\nu^0_{fr}}{\nu^0_{fr} + \nu^0_{mr}}\left[1 + \frac{\nu^0_{mr}}{\nu^0_{fr}}\, e^{-(\nu^0_{fr}+\nu^0_{mr})x}\right] \quad L_m{}^f = \frac{\nu^0_{mr}}{\nu^0_{fr} + \nu^0_{mr}}\left[1 - e^{-(\nu^0_{fr}+\nu^0_{mr})x}\right]$$

$$L_F{}^f = \frac{\nu^0_{mr}}{\nu^0_{fr} + \nu^0_{mr}}\left[1 + \frac{\nu^0_{fr}}{\nu^0_{mr}}\, e^{-(\nu^0_{fr}+\nu^0_{mr})x}\right] \quad L_F{}^m = \frac{\nu^0_{fr}}{\nu^0_{fr} + \nu^0_{mr}}\left[1 - e^{-(\nu^0_{fr}+\nu^0_{mr})x}\right] \quad (32)$$

where the superscript zero on the ν's indicates the zero dose values of the transition densities. Note that in the special case in which there are no lethal lesions $L_M{}^m + L_M{}^f = 1$ and $L_F{}^f + L_F{}^m = 1$; however, these sums are not equal to unity in the general case, as the presence of lethal lesions causes this sum to decrease as x increases.

APPENDIX B

MARKER PRESENCE

The most general forms of the marker presence relationships are those which include the possibility of lethal lesions induced on the female chromosome by irradiation. Expressions for $R(a, \bar{s}; D_f)$ and $R(a, b, \bar{s}; D_f)$ are given in equations 22 and 23. (Equations 9 and 10 are special cases of these in which $(P_l(x) = e^{-\nu_{fl}x} = 1$; i.e., where $\nu_{fl} = 0$.)

To perform the required integrations, proper substitutions for the association functions (equations 31) are made in equations 22 and 23, using the relationships $P_l(x) = e^{-\nu_{fl}x}$ and $P_t(x) = e^{-\nu_{mt}x}$. We obtain after integration

$$R(a, \bar{s}; D_f)/N(0) = K\{L_F{}^m(x_a)I_1(x_a) + L_F{}^m(x_a)L_m{}^f(x_s - x_a)I_2 + {}_mL^f(x_s - x_a)L_F{}^m(x_E - x_s + x_a)P_l(x_E)\}$$

and

$$R(a, b, \bar{s}; D_f)/N(0) = K\{L_F{}^m(x_a)L_M{}^m(x_b - x_a)I_1(x_b) + L_F{}^m(x_a)L_M{}^m(x_b - x_a)L_M{}^f(x_s - x_b)I_2 + L_M{}^m(x_b - x_a)L_M{}^f(x_s - x_b)L_F{}^m(x_E - x_s + x_a)P_t(x_E)\} \quad (33)$$

where

$$I_1(x_i) = \int_{x_i}^{x_s} L_M{}^f(x - x_i)P_l(x_E - x)P_t(x)\nu_{mt}\,dx$$

$$= \frac{\gamma}{2}\frac{\nu_{mr}\nu_{mt}}{\nu_{mt} + \alpha - \nu_{fl}} e^{-\nu_{mt}x_i}e^{-\nu_{fl}(x_E - x_i)}[1 - e^{-(\nu_{mt}+\alpha-\nu_{fl})(x_s - x_i)}]$$

$$- \frac{\gamma}{2}\frac{\nu_{mr}\nu_{mt}}{\nu_{mt} + \beta - \nu_{fl}} e^{-\nu_{mt}x_i}e^{-\nu_{fl}(x_E - x_i)}[1 - e^{-(\nu_{mt}+\beta-\nu_{fl}(x_s - x_i)}] \quad (34)$$

$$I_2 = \int_{x_s}^{x_E} L_F{}^f(x - x_s)P_l(x_E - x)P_t(x)\nu_{mt}\,dx$$

$$= \frac{1 - \delta}{2}\frac{\nu_{mt}}{\nu_{mt} + \alpha - \nu_{fl}} e^{-\nu_{mt}x_s}e^{-\nu_{fl}(x_E - x_s)}[1 - e^{-(\nu_{mt}+\alpha-\nu_{fl})(x_E - x_s)}]$$

$$+ \frac{1 + \delta}{2}\frac{\nu_{mt}}{\nu_{mt} + \beta - \nu_{fl}} e^{-\nu_{mt}x_s}e^{-\nu_{fl}(x_E - x_s)}[1 - e^{-(\nu_{mt}+\beta-\nu_{fl})(x_E - x_s)}]. \quad (35)$$

GENETIC ANALYSIS OF UNSELECTED PROXIMAL MARKERS

Low dose. Assume

$$e^{-\nu_{mt}(x_s - x_b)} \ll 1; \qquad e^{-(\beta-\alpha)x_a} \ll 1$$

$$R(b, \bar{s}; D_f)/N(0) = KL_F{}^m(x_b)I_1(x_b)$$

$$R(a, b, \bar{s}; D_f)/N(0) = KL_F{}^m(x_a)L_M{}^m(x_b - x_a)I_1(x_b)$$

$$\therefore G(a \mid b, \bar{s}; D_f) = \frac{R(a, b, \bar{s}; D_f)}{R(b, \bar{s}; D_f)} = \frac{L_F{}^m(x_a)}{L_F{}^m(x_b)} L_M{}^m(x_b - x_a)$$

or,

$$G(a \mid b, \bar{s}; D_f) \approx \frac{1}{2}(1 + \delta)\left[1 + \frac{1 - \delta}{1 + \delta} e^{-(\beta-\alpha)(x_b - x_a)}\right] \quad (28)$$

by the use of equations (31).

At zero dose.

$$G(a \mid b, \bar{s}; 0) = \frac{\nu_{fr}^0}{\nu_{fr}^0 + \nu_{mr}^0}\left[1 + \frac{\nu_{mr}^0}{\nu_{fr}^0} e^{-(\nu_{mr}^0 + \nu_{fr}^0)(x_b - x_a)}\right]. \quad (13)$$

High dose.

$$G(a \mid b, \bar{s}; D_f) = \frac{L_F{}^m(x_E - x_s + x_a)}{L_F{}^m(x_E - x_s + x_b)} L_M{}^m(x_b - x_a)$$

$$\approx \frac{1}{2}(1 + \delta)\left[1 + \frac{1 - \delta}{1 + \delta} e^{-(\beta-\alpha)(x_b - x_a)}\right]. \quad (28)$$

Low dose. Assume

$$e^{(-\nu_{mt}+\alpha-\nu_{ft})(x_s-x_b)} \ll 1; \qquad e^{-(\beta-\alpha)x_a} \ll 1$$

$$R(a, \bar{s}; D_f)/N(0) = KL_F{}^m(x_a)I_1(x_a)$$

$$R(a, b, \bar{s}; D_f)/N(0) = KL_F{}^m(x_a)L_M{}^m(x_b - x_a)I_1|(x_b)$$

$$\therefore G(b \mid a, \bar{s}; D_f) = L_M{}^m(x_b - x_a)\frac{I_1(x_b)}{I_1(x_a)}$$

$$\approx \frac{1}{2}(1+\delta)e^{-(\nu_{mt}+\alpha-\nu_{ft})(x_b-x_a)}\left[1 + \frac{1-\delta}{1+\delta}e^{-(\beta-\alpha)(x_b-x_a)}\right]. \quad (30)$$

Zero dose.

$$G(b \mid a, \bar{s}; 0) = \frac{\overset{0}{\nu}_{fr}}{\overset{0}{\nu}_{fr} + \overset{0}{\nu}_{mr}}\left[1 + \frac{\overset{0}{\nu}_{mr}}{\overset{0}{\nu}_{fr}}e^{-(\overset{0}{\nu}_{fr}+\overset{0}{\nu}_{mr})(x_b-x_a)}\right]e^{-\nu_{mt}(x_b-x_a)}$$

$$= L_M{}^m(x_b - x_a)P_t(x_b - x_a).$$

High dose.

$$G(b \mid a, \bar{s}; D_f) = \frac{L_M{}^f(x_s - x_b)}{L_M{}^f(x_s - x_a)}L_M{}^m(x_b - x_a)$$

$$= \frac{1}{2}(1+\delta)\left[1 + \frac{1-\delta}{1+\delta}e^{-(\beta-\alpha)(x_b-x_a)}\right]. \quad (28)$$

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