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Effects on genetic recombination of *Escherichia coli* K 12 produced by P^{32} decay in the Hfr male*

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1. INTRODUCTION

Fuerst *et al.* (1956) have shown that the decay of incorporated P^{32} in the Hfr H strain of *E. coli* K 12, prior to bacterial conjugation, differentially inhibits the appearance of Hfr genetic characters in recombinants. By measuring the slopes of the experimental 'suicide' curves for the appearance of various markers as a function of prior P^{32} dose they were able to construct a map of the first quarter of the Hfr H chromosome. It was found that the relative distances between genetic markers so measured were approximately the same as the distances determined independently by interrupted mating experiments. Similar results have been obtained for the same Hfr strain over a considerably larger chromosomal region by Marcovich (1961) using X-irradiation of the donor cells prior to mating, rather than P^{32} decay.

These results have been interpreted as indicating that P^{32} decay, or X-irradiation, produces randomly located lesions in the Hfr H chromosome which lead to the interruption of genetic transfer during mating. There is indirect evidence from a variety of sources that P^{32} decay in bacteria and bacteriophages can result in complete chromosomal breaks and that these breaks are the primary lethal lesions. (For a review, see Stent & Fuerst, 1960.) It has been found also that in Hfr H the efficiency with which chromosomal P^{32} decays cause interruptions of chromosomal transfer is of the same order of magnitude as the efficiency for lethalities. It has therefore been proposed that both the genetic and lethal effects of P^{32} decay in Hfr H are due to those radioactive disintegrations which lead to complete chromosomal breaks. It should be noted that a variety of recent work has indicated that the mechanism of P^{32} lethality may be more complex than originally believed and that there may be intermediate steps, probably chemical, between the actual radioactive disintegration and the ultimate lethal event. (For further discussion and references, see Yan & Kondo, 1964.)

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In the current study the methods of Fuerst *et al.* have been used to compare the genetic effects of P^{32} decay before mating in two different Hfr strains, Hfr H, previously studied, and Hfr Cavalli (Hfr CS), which transfers its chromosome in the opposite direction. Genetic and lethal effects have been compared within each strain and we have studied the influence of temperature and phase state during storage on P^{32} effects in Hfr H.

Finally, in order to more fully assess the significance of this kind of radiation mating experiment in distinguishing among current models for chromosomal transfer during bacterial conjugation, the qualitative arguments proposed by Jacob *et al.* (1963) have been put into quantitative form.

2. MATERIALS AND METHODS

(i) *Strains*

Escherichia coli K 12. The order of transfer of utilized markers by Hfr Hayes is: *0*, *thr*$^+$, *leu*$^+$, *lac*$^+$, *gal*$^+$, *try*$^+$, *his*$^+$, *str*^{s}. The order of transfer of markers by Hfr Cavalli (obtained from Dr A. Garen) is: *0*, *lac*$^+$, *leu*$^+$, *thr*$^+$, *arg*$^+$, *xyl*$^+$, *str*^{s}. F$^-$ strain PA-309 carries the markers *thr*$^-$, *leu*$^-$, *lac*$^-$, *gal*$^-$, *try*$^-$, *his*$^-$, *str*^{r}, *xyl*$^-$, *arg*$^-$, *thi*$^-$.

(ii) *Media*

Growth medium was the H medium of Stent & Fuerst (1955) modified by the omission of glycerol and the addition of 3 mg./l. of separately sterilized thiamine. The total phosphorus concentration was varied as desired by making up the H medium at twice its final concentration and diluting with an equal volume of NaH_2PO_4 solution of the proper concentration. Storage medium (modified after McFall *et al.* 1958): K_2HPO_4 7 g., sodium citrate 0·5 g., $MgSO_4.7H_2O$ 0·1 g. $(NH_4)_2SO_4$ 1 g., KH_2PO_4 2 g., glycerol 100 ml., distilled water to a total volume of 1 l. The selective solid media were very similar to those described by Wood & Marcovich (1964).

(iii) *Radioactive techniques*

Techniques were generally similar to those originally described by Stent & Fuerst (1955). Radiophosphorus was obtained as carrier free $H_3P^{32}O_4$ from Oak Ridge National Laboratory, Oak Ridge, Tennessee. This P^{32} solution was evaporated to dryness by means of an infrared lamp and resuspended in a NaH_2PO_4 solution of the desired concentration. This solution was sterilized and used to make up radioactive H medium by mixing in equal parts with double strength H medium. Measurements of radioactivity were made on dried samples by means of a thin window GM tube. For the P^{32} labelling procedure, a culture of the desired cells was grown overnight in ordinary H medium and inoculated (1:200) into radioactive H medium. This culture tube was then agitated at 37°C. long enough to allow a fifty-fold increase in cell number, giving a viable cell count of about 10^8 cells/ml. After growth the radioactive cells were filtered (0·45 μ pore size

membrane filter), washed several times with buffer solution, and resuspended in chilled storage medium. For storage of cells during decay of P^{32}, 0·2–0·3 ml aliquots of this suspension were placed in glass vials (10 × 40 mm.) which were immediately sealed and placed in liquid nitrogen at −196°C.

(iv) *Mating and assay*

Female cells for each mating were grown in H medium to a concentration of $1–2 \times 10^8$ cells/ml. (exponential phase). Radioactive male cells were rapidly thawed in a 45°C. water bath and 0·2 ml. of this suspension and 1·8 ml. of the female culture were mixed and incubated at 37°C. for the desired interval with gentle shaking. After mating, aliquots were diluted and plated on recombinant assay plates, which were incubated for 2 days before reading. The genetic analysis of recombinants for unselected male characters was carried out by inoculation and regrowth of recombinant colonies onto a grid plate, followed by the use of a printing technique (Lederberg & Lederberg, 1952) with the desired test plates. Each day's matings were carried out in duplicate, with male cells from different vials and female cells from different growth tubes. Genetic analyses involved two grid plates from each of the duplicate matings, or a total of 200 colonies per dose point, for a given selected marker.

3. RESULTS

(i) *Effects of P^{32} decay on recombinant formation and Hfr survival*

Fig. 1 shows representative experiments for both the Hayes and Cavalli strains illustrating the effects of P^{32} decay in the Hfr cell before mating. All experimental conditions were similar except that 90-min. matings were used for Hfr H and 60-min. matings for Hfr CS. The different durations were used because the streptomycin marker used for counterselection is transferred much earlier in matings involving Hfr CS. Hfr survival curves, based on assays just before mating, are included for comparison with 'marker survival' curves in Fig. 1.

In Table 1 are our best estimates for marker distances from the Hfr origin, determined from the slopes of curves such as those in Fig. 1. In measuring the slopes, zero-dose points were ignored because of possible initial shoulders on some of the curves. To convert relative slopes to equivalent marker distances from the origin, *try*$^+$ was normalized to a distance of 32 min. for Hfr H and *arg*$^+$ was normalized to 32 min. for Hfr CS, the proper entry times for these markers according to data from interrupted mating experiments. These two markers were chosen for normalization because each is located at the same intermediate distance from its respective chromosomal origin. Most of the P^{32} equivalent distances in Table 1 are based on data from two experiments, and include an associated experimental uncertainty.

Marker distances from the origin for each strain as determined by interrupted mating experiments are also included in Table 1 for comparison with the P^{32}

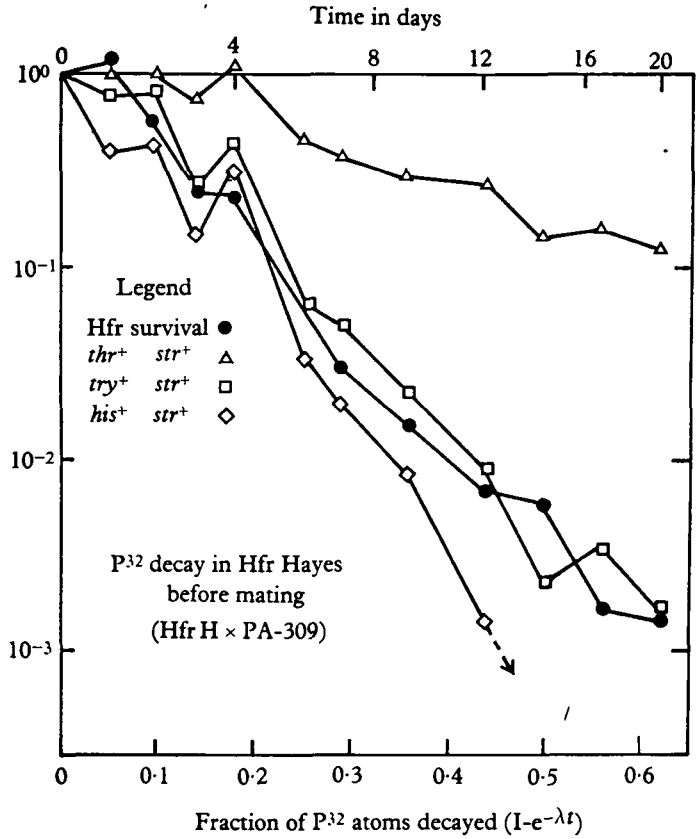

Fig. 1*a*. Appearance of selected markers of Hfr H ($A_0 = 67$ mc./mg.) among recombinants, when radioactive decay proceeds in donor cells stored at −196°C. before conjugation for 90 min. with non-labelled PA-309 recipient bacteria. The data is normalized in each case to the zero-dose number of recombinants. Hfr survival, similarly normalized, is plotted for cells assayed just before mating.

distances. The distance for the earliest marker for each strain is taken to be the measured entry time for matings involving that strain, while genetic distances between markers are based on the recent work of Taylor & Thoman (1964), involving composite data from many strains. While some of these genetic distances, notably the *try–his* distance, are considerably shorter than previously published distances, experiments in this laboratory, involving Hfr strains which transfer the markers of interest early, consistently support their accuracy. The uncertainties associated with these distances were determined by assuming that all of the relative distances are known to an accuracy of better than 10%, while the uncertainty of the entry time for the first marker of each strain is about 2 min. The question of the validity of using entry times for early markers to represent genetic distances from the chromosomal origin will be discussed later.

It can be seen from Table 1 that there is good agreement between the equivalent marker distances from the origin determined by the present P^{32} experiments and

marker distances determined from interrupted mating experiments, with the assumption that the entire mating time represents chromosomal transfer. In addition, the relation between the sensitivity to P^{32} decay of Hfr survival and of

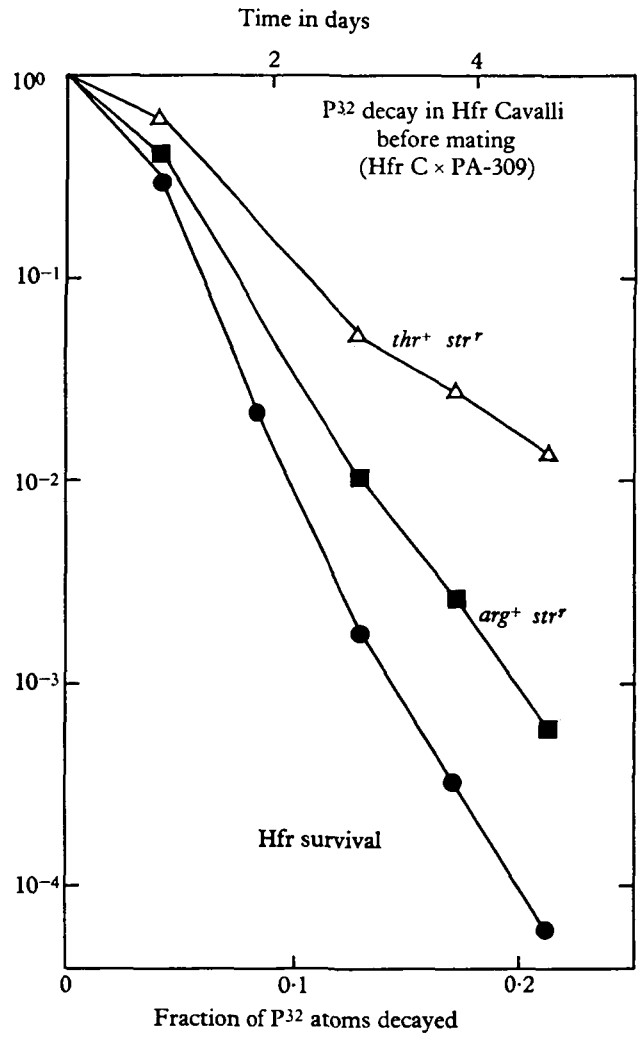

Fig. 1*b*. Appearance of selected markers of P^{32} labelled Hfr CS ($A_0 = 103$ mc./mg.) among recombinants assayed after conjugation for 60 min., as well as Hfr survival. Details generally similar to those of Fig. 1*a*.

recombinant formation appears to be similar for each of the two Hfr strains. The Hfr survival curve has, on the average, the same slope as a recombinant 'survival' curve for which the selected male marker is 32 min. from the origin in the case of Hfr H and 40 min. for Hfr CS, with overlapping intervals of uncertainty.

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Table 1. *Comparison of chromosome maps for Hfr H and Hfr CS constructed on the basis of marker sensitivity to P^{32} decay and on the basis of data from interrupted matings*

(*a*) *Hfr Hayes*

	thr	*try*	*his*	Hfr survival
P^{32} marker distance from origin in minutes, normalized to *try* = *32*	9·5 ± 2	32	43 ± 5	32 ± 4
Marker distance from origin in minutes, from interrupted matings	8 ± 2	32 ± 3	47 ± 4	—

(*b*) *Hfr Cavalli*

	lac	*thr*	*arg*	*xyl*	Hfr survival
P^{32} marker distance from origin in minutes, normalized to *arg* = 32	8·4 ± 2	21 ± 3	32	46 ± 5	40 ± 5
Marker distance from origin in minutes, from interrupted matings	10 ± 2	20 ± 2	32 ± 3	39 ± 4	—

Control experiments were carried out with both Hfr strains, using unlabelled cells stored in highly radioactive medium, calculated to give the approximate dose of external beta irradiation received by stored cells in the above experiments. No significant decrease was found either in Hfr survival or in recombinant formation in these control experiments.

(ii) *Effects of P^{32} decay on inheritance of unselected markers*

In Fig. 2 the recombinants selected in mating experiments of the kind illustrated in Fig. 1 have been analyzed for the inheritance of unselected prototrophic characters from the male parent. In each diagram the fractional occurrence of various unselected markers in a typical recombinant type is shown as a function of P^{32} dose received by the Hfr prior to mating. Several important features stand out which were consistently reproducible, in spite of the considerable statistical fluctuations in results for markers appearing with low frequencies. Unselected male markers between the origin and the selected marker appear to be independent of dose in their frequency of appearance, the frequency varying between 40% and 90%, depending on the genetic distance between the selected and unselected markers in question, i.e., the linkage. As would be expected from the 'marker survival' curves, the appearance of unselected markers distal to the selected marker falls off exponentially with the product of P^{32} dose and the genetic distance between the selected and unselected markers. These results hold for matings involving either Hfr strain.

(iii) *Effects of storage temperature on P^{32} sensitivity*

To study the relation of temperature and phase state during storage to the lethal and genetic effects of P^{32} decay, mating experiments were carried out with

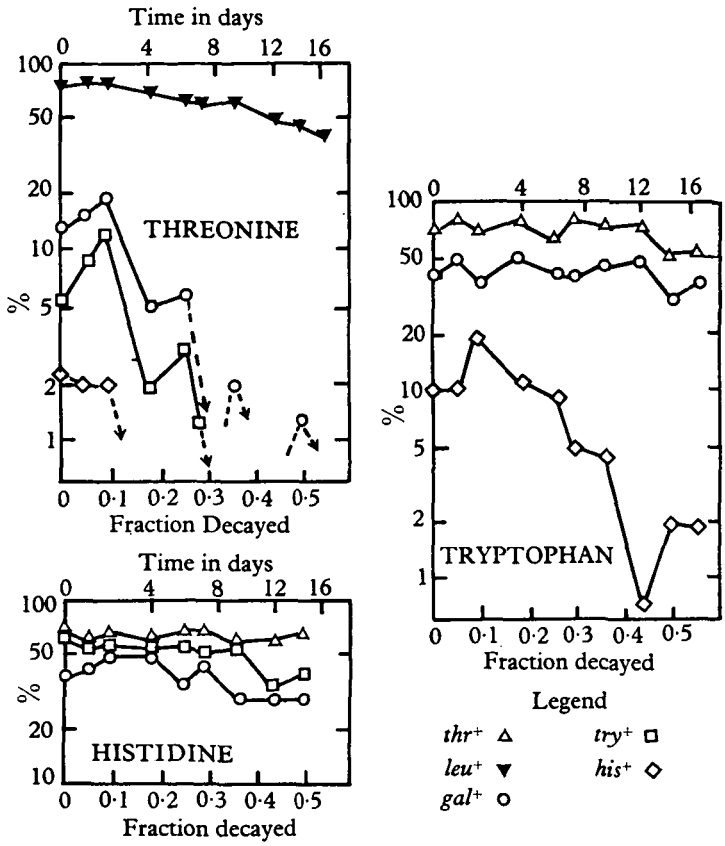

Fig. 2*a*. Effects of P^{32} decay in the Hfr on the inheritance of unselected male markers by recombinants. Each graph shows the proportion of recombinants selected for a given character which inherit various unselected characters as a function of P^{32} dose prior to mating. Matings involve Hfr H ($A_0 = 47$ mc./mg.) and non-labelled PA-309.

Hfr H cells stored under various conditions before mating. P^{32} labelled cells were stored in the frozen state at -10°C. after initial freezing at -196°C. or in the liquid state at -2°C. without any freezing. The results have been compared with those of experiments where freezing and storage were at -196°C. Control experiments showed that storage of non-radioactive cells at either -10°C. or -2°C. caused some progressive decrease in cell viability and recombinant formation. Corrections were made for these storage effects before comparison was made with experiments involving liquid nitrogen storage.

We find that Hfr H survival and the formation of *thr*$^+$, *try*$^+$, and *his*$^+$ recombinants are all 2·5–3·0 times more sensitive to P^{32} decay when Hfr storage is at temperatures near 0°C. than when storage is at -196°C. Genetic analyses also showed this same increased sensitivity to P^{32} effects at higher storage temperatures. Whether storage was in the frozen (-10°C.) or liquid (-2°C.) state did not make a significant difference in sensitivity to any of the P^{32} effects studied.

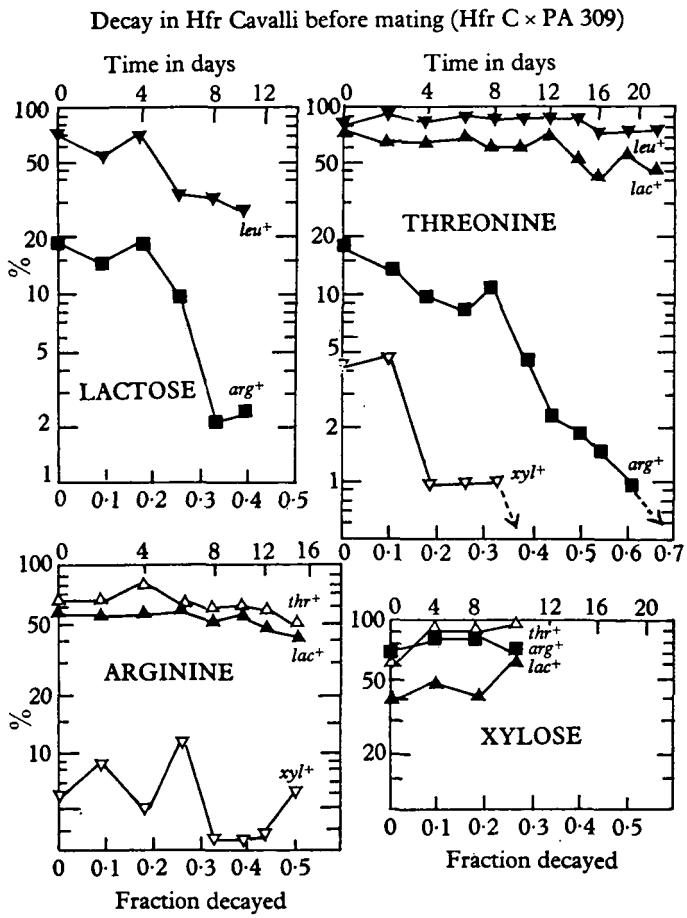

Fig. 2*b*. Experiments analogous to those of Fig. 2*a*, with matings involving Hfr CS ($A_0 = 45$ mc./mg.) and non-labelled PA-309.

4. DISCUSSION

(i) *Lethal and genetic effects of* P^{32} *decay in* E. coli *K 12, Hfr*

The dose–response curves for the inheritance of selected male characters by recombinants in the present study can be most simply expressed by the relation $N/N_0 = e^{-\alpha x D}$, where N/N_0 is the number of recombinants of a particular type divided by the zero-dose number, α is the P^{32} sensitivity constant of the Hfr strain, x is the genetic distance from the chromosomal origin to the selected Hfr character, and D is the P^{32} dose to the male cell prior to mating. This relationship has been found to hold true within the limits of experimental error for genetic markers of Hfr H as far as 47 min. from the origin. The same relation is also valid when P^{32} decay occurs in Hfr CS before mating. The previous work of Fuerst *et al.* (1956), involving a study only of markers on Hfr H within the first 24 min. from the origin, is also compatible with this dose-response relationship. The results of Marcovich

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(1961) for X-irradiation of Hfr H before mating can also be expressed by an analogous relationship. The similarity of our results for the two strains indicates that the genetic effects of P^{32} decay on bacterial conjugation are not peculiar to Hfr H and are not dependent either on the location of the Hfr origin or on the direction of chromosomal transfer, since the strains have different origins and transfer their chromosomes in opposite directions.

In Fig. 1 and Table 1 we saw that the survival curves for both Hfr H and Hfr CS could be roughly superimposed on a dose–response curve for the appearance of recombinants inheriting a male marker approximately one-third of the total chromosomal length away from the origin. This is the same relation previously reported for Hfr H alone in experiments involving P^{32} decay (Stent & Fuerst, 1960) or X-irradiation (Marcovich, 1961) before mating. The simplest interpretation of this finding is that approximately one-third of these P^{32} disintegrations or X-ray lesions in the DNA of the Hfr cell which are capable of interrupting chromosomal transfer also cause lethalities, as determined by loss of colony-forming ability. It is not clear why the two effects have different sensitivities if each is due to those decays which lead to double strand chromosomal breaks, although the discrepancy can be easily explained in terms of hypothetical repair mechanisms.

The fact that genetic and lethal effects of P^{32} decay in Hfr H showed the same temperature dependence is consistent with the concept of a common mechanism for both effects. The temperature variation of these effects by a factor of 2·5–3·0 between storage at -196°C. and temperatures near 0°C. is fairly close to that reported by Stent & Fuerst (1955) for a wide variety of bacteriophages. These showed approximately twice the sensitivity to the lethal effects of the decay of incorporated P^{32} at $+4$°C. as at -196°C. All of these similarities are consistent with a common mechanism for lethal and genetic effects of incorporated P^{32} in both bacteria and bacteriophages.

Our studies on the inheritance of unselected Hfr markers as a function of P^{32} dose gave essentially the same results reported by Marcovich for X-irradiation of Hfr H before mating, again demonstrating the similarity in genetic effects of these two different modes of radiation treatment. For both Hfr strains in the present study, the inheritance by recombinants of unselected male markers between the origin and the selected male marker did not change as a function of P^{32} dose. This indicates that those radioactive disintegrations in the DNA which do not halt chromosomal transfer (at least 90%) have no other detectable genetic effects on recombinants. Our findings with respect to the P^{32} sensitivity of the inheritance of unselected male markers distal to the selected marker confirm and extend those of Fuerst *et al.* for the P^{32} sensitivity of the inheritance of several distal male markers by *thr*$^+$ *leu*$^+$ recombinants of Hfr H. Both their results and ours indicate that the inheritance of such unselected distal markers is sensitive to dose approximately according to the same relationship found for the inheritance of selected male markers by recombinants, $N/N_0 = e^{-\alpha x D}$, except that now x refers to the genetic distance from the marker of interest to the selected marker, rather than the distance to the origin as before.

(ii) *Significance for current models of chromosomal transfer*

There are at least three theories of chromosomal transfer by the Hfr donor cell which make definite predictions for experiments involving P^{32} labelled Hfr cells. We shall discuss the predictions of each of these theories for the dose dependence of the inheritance of selected male markers by recombinants.

Perhaps the simplest theory is that following an effective contact between mating partners, the circular Hfr chromosome breaks at the origin and is somehow pushed sequentially into the female cell, independently of any DNA synthesis. This proposal predicts that a break in the Hfr chromosome, whether due to P^{32} decay or any other cause, will simply halt chromosome transfer at the breakage point. If one assumes random chromosomal breakage due to uniform P^{32} labelling of the chromosome and a single-hit, single-target mode of action, then a dose–response curve for the inheritance of selected Hfr characters by recombinants of the form $N/N^0 = e^{-\alpha x D}$ is predicted, where the terms are defined as for the analogous experimental relationship. Furthermore, this theory immediately accounts for the experimentally determined variation with marker distance from the origin of the zero-dose frequencies of recombinants ($N_0 = Ce^{-kx}$) in terms of random chromosomal breaks induced by factors other than radiation.

The recently proposed replicon theory of Jacob *et al.* (1963) assumes that effective mating contact serves to initiate both the transfer of the origin and a new round of DNA synthesis starting at the origin and proceeding towards the end of the chromosome which is transferred last (the distal end). In this theory it is the hybrid chromosome which is transferred as it is synthesized. Like the first theory, this one predicts that a chromosomal break due to P^{32} decay will serve only to halt the transfer of further chromosomal material, in this case by stopping DNA replication at the site of the break; thereby resulting again in a dose–response curve for the appearance of recombinants of the form $N/N_0 = e^{-\alpha x D}$.

It should be noted that for each of these two theories the same arguments used to derive the dose dependence of the inheritance of selected male markers can be applied to the case of distal unselected male markers, giving the same dose–response relationship, with x representing the genetic distance from the selected marker to the unselected marker of interest. Since the experimental data is rather poor for unselected male markers, this agreement is less conclusive.

In the other current theory of chromosomal transfer, proposed by Bouck & Adelberg (1963), it is assumed that replication of the Hfr chromosome always proceeds from the distal end to the origin. If an effective contact is made with a recipient cell, the round of DNA replication under way in the circular Hfr chromosome must be completed before genetic transfer can begin. Transfer is initiated when DNA replication has reached the origin and the circular chromosome is momentarily broken there. This free origin passes through the mating tube and the Hfr chromosome follows sequentially, until transfer is interrupted.

The predictions of this theory for the results of mating experiments involving prior P^{32} decay or X-irradiation of the Hfr cells are not immediately evident.

On the basis of a qualitative discussion alone, Jacob *et al.* (1963) suggested that this theory is incompatible with the P^{32} results of Fuerst *et al.* unless various *ad hoc* assumptions are made. It will be informative to discuss the predictions of this theory quantitatively. To do so we make the following simplifying assumptions. We assume that the P^{32} labelled cells are unsynchronized and exponentially growing when frozen in liquid nitrogen, and that the replication time of the DNA molecule is the same as the cell division time. This means that the replicating fork will be located at random points along the Hfr chromosome in the frozen population. We also assume that P^{32} decay causes randomly located chromosomal breaks, and that if such a break is located between the origin and the replicating fork it will prevent the completion of the current round of DNA replication when the cells are thawed, and therefore prevent the initiation of genetic transfer upon

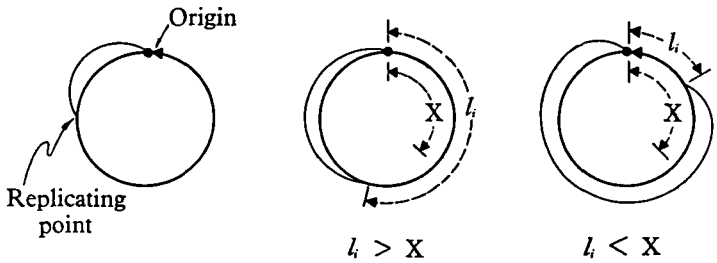

Fig. 3. Diagrammatic representation of three stages in the replication of the circular Hfr chromosome.

mating. Finally, we assume that a break in one of the two copies of the chromosome distal to the replicating fork will not halt DNA replication in the proximal region, but will stop the transfer of that copy of the chromosome at the point of breakage.

We are now ready to study the variation with prior radiation dose to the male of the number of recombinants inheriting an Hfr character at a genetic distance x from the origin. Let $N(D,x)$ be the total number of such recombinants appearing subsequent to a dose D; and let $n_i(D,x)$ be the number of such recombinants arising from Hfr cells frozen with the replicating DNA fork in the small interval $l_i \pm \epsilon$ from the origin, where ϵ is some length very small compared to the total chromosomal length L. The total number of recombinants, $N(D,x)$ is the sum over all values of i for which $0 \leqslant l_i \leqslant L$:

$$N(D,x) = \sum_{l_i=0}^{l_i=L} n_i(D,x) \tag{1}$$

We now apply our previous assumptions to calculate $n_i(D,x)$ for the two situations illustrated in Fig. 3, where $l_i > x$, or $l_i < x$. If $l_i > x$, the formation of recombinants inheriting the marker at x will be prevented whenever there is a radiation break in the region between the origin and the small interval at a distance l_i away, regardless of the exact value of x, so that if n_{0i} is the number of recombinants

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formed with zero dose, then:

$$n_i(D,x) = n_{0i}e^{-\alpha l_i D} \tag{2}$$

If $l_i < x$, a recombinant cannot appear unless the current round of DNA replication in the Hfr is completed and, in addition, no breaks are present in the chromosome between distances l_i and x from the origin. Then for $l_i < x$:

$$n_i(D,x) = n_{0i}\, e^{-\alpha l_i D}\, e^{-\alpha(x-l_i)D} \tag{3}$$

Simplifying this term and adding the contributions for all of the cells in the population, we get:

$$N(D,\ x) = \sum_{l_i=0}^{l_i=x} n_{0i}e^{-\alpha x D} + \sum_{l_i=x}^{l_i=L} n_{0i}e^{-\alpha l_i D} \tag{4}$$

Now we go to the continuous limit and substitute $l_i = l$, $n_{0i} = N(0,x)\ dl/L$, giving:

$$\frac{N}{N_0} = \frac{N(D,x)}{N(D,0)} = \frac{1}{L}\int_0^x e^{-\alpha x D}\,dl + \frac{1}{L}\int_x^L e^{-\alpha l D}\,dl \tag{5}$$

The final result is:

$$\frac{N}{N_0} = \left(\frac{x}{L} + \frac{1}{\alpha LD}\right)e^{-\alpha x D} - \frac{1}{\alpha LD}\,e^{-\alpha L D} \tag{6}$$

In Fig. 4 the theoretical relation of equation (6) has been used to construct curves for the appearance of recombinants inheriting selected male markers at various distances from the origin, as a function of P^{32} decay or radiation dose to the male prior to mating, in analogy to Fig. 1. Numerical data corresponding approximately to the experimental P^{32} doses and genetic distances of Fig. 1*a* were substituted into equation (6), assuming a total chromosomal length of 90 min.

We see from Fig. 4 that the predictions of the model of Bouck and Adelberg are markedly different from those of the other two models only for low radiation doses ($N/N_0 > 0{\cdot}1$). In keeping with the suggestion of Jacob *et al.*, the 'inactivation curves' for various male markers are relatively independent of chromosomal location in this dose range. However, at higher doses the inactivation for all male markers except those very close to the origin become nearly straight lines with slopes closely proportional to marker distance from the origin. For markers close to the origin, the slopes are greater than predicted by the other two theories.

Thus, the opportunities for clearly distinguishing between the model of Bouck and Adelberg and the other two models by this kind of radiation mating experiment involve the examination of marker inactivation curves at low radiation doses and the study of male markers very close to the origin. Comparison of theory with experiment at low radiation doses is complicated by the possibility that the genetic effects under study may actually be due to cumulative radiation action, whether multi-hit or multi-target. If this were the case, the marker inactivation

curves would deviate from any of the theoretical curves derived here for single-hit, single-target effects. The deviation would be important chiefly at low doses and would involve a tendency towards an initial upward convexity in all of the inactivation curves. Even if the theory of Bouck and Adelberg for genetic transfer were entirely correct, the effect of cumulative radiation action could easily outweigh the initial upward concavity seen in Fig. 4. Some of our marker inactivation curves showed a slight initial upward convexity, as did some of those presented by Fuerst *et al.*, suggesting that cumulative action may play a role in these radiation effects.

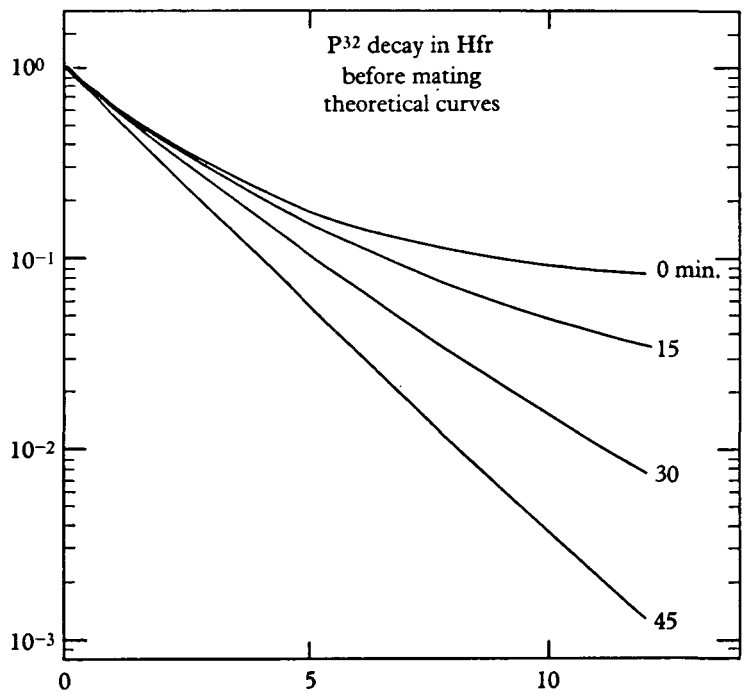

Fig. 4. Theoretical effects of P^{32} decay in the Hfr on the inheritance of selected male markers by recombinants, based on the theory of Bouck and Adelberg for genetic transfer. Maximum marker distances and radiation doses have been chosen to correspond approximately to those of Fig. 1*a*. The distance in minutes of the selected male marker from the origin is shown for each curve.

There is also great difficulty in using the experimental P^{32} inactivation curves for male markers near the origin to rule out the model of Bouck and Adelberg. Since no marker of any Hfr strain appears to be transferred with an entry time of less than 5 min., regardless of its chromosomal location, there is a real possibility that as much as the first 5 min. of mating time may represent not chromosomal transfer but preliminary stages of conjugation. If this is the case, then the proportionality reported here and elsewhere between marker distance from the origin and P^{32} sensitivity is not true for markers transferred very early, because they

are relatively much closer to the chromosomal origin than is indicated from the measured times of transfer. The theory of Bouck and Adelberg actually predicts such a disproportion between marker distance from the origin and radiation sensitivity for male markers close to the origin.

In summary, it is clear that with the simplifying assumptions made the theory of Bouck and Adelberg makes predictions concerning the results of P^{32} mating experiments which are different from the predictions of the other two theories discussed. However, the differences are much less striking than those anticipated by Jacob *et al.* on the basis of qualitative arguments alone. It is true that the experimental data presented here and elsewhere seems to be more readily compatible with exponential marker inactivation curves with slopes proportional to genetic distances from the origin, and therefore with either of the first two models of genetic transfer. Nevertheless, in the light of the problems discussed in the last two paragraphs it would seem most premature to attempt to rule out the theory of Bouck and Adelberg on the basis of radiation mating experiments.

SUMMARY

The effects of P^{32} disintegration in the Hfr donor cell on the genetic recombination of *Escherichia coli* K 12 have been studied. The dose–response relationship for the transfer of markers at a distance x from the chromosomal origin, $N/N_0 = e^{-\alpha x D}$, which summarizes the findings of Fuerst *et al.* (1956) for early markers of Hfr H, has been found to hold true to a good approximation for more distal selected markers in this strain, as well as for selected markers of Hfr CS, which transfers its chromosome in the opposite direction. The inheritance by recombinants of non-selected male markers located between the origin and the selected marker is not affected at all by prior P^{32} dose to the Hfr. The sensitivity of each Hfr strain studied to the lethal effects of P^{32} decay corresponds to the sensitivity to transfer effects of a male marker located about one-third of the total chromosomal length away from the origin. Genetic and lethal effects in Hfr H showed the same dependence on storage temperature during P^{32} decay. Our data, therefore, supports the concept of a common origin for the lethal and genetic effects of P^{32} decay.

The quantitative predictions of current models for chromosome transfer with regard to radiation mating experiments have been examined. A simple model, involving no DNA synthesis, as well as the replicon model of Jacob *et al.* (1963) predict exponential marker inactivation curves of the kind found experimentally. The model of Bouck & Adelberg (1963) predicts a more complex dose dependence, but one not nearly so different from that of the other two models as was anticipated by Jacob *et al.* It appears unjustified to attempt to exclude any of the three models on the basis of presently available data from radiation mating experiments.

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