# AUTORADIOGRAPHIC STUDY OF TRANSFER OF DNA DURING BACTERIAL CONJUGATION

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ABSTRACT Amounts of $H^3$-thymine–labeled DNA that are transferred from cell to cell in Hfr × $F^-$ and $F^+$ × $F^-$ matings have been measured autoradiographically. The transferred amounts are compared to the DNA content of the bacterial chromosome, which was measured autoradiographically on the basis of a model for the replication of DNA in *E. coli* (1). Partition of transferred radioactivity among the progeny of $F^-$ exconjugants was also observed by autoradiography. When $F^+$ donors were used, it was found that the transferred label often remained intact during growth of the recipients into microcolonies. The class of colonies in which this occurred was shown to contain a uniform amount of radioactivity amounting to 0.5 to 0.9 per cent of the bacterial chromosome. Dispersal of the radioactivity into smaller units was also observed. The fraction of females receiving F from $F^+$ donors, as measured genetically, was close to the fraction of females receiving DNA-label. The distributions of amounts of radioactivity transferred to females by Hfr males after different periods of mating were shown to fit the predictions of genetic analysis of chromosomal transfer—on the assumption that the total genetic map corresponds to the whole bacterial chromosome. The label transferred by Hfr donors was found to be variably dispersed among the progeny (4 to 5 generations) of the recipient cells.

## INTRODUCTION

On the basis of genetic experiments it has been concluded that most of the genetic determinants of *E. coli* K12 are linearly arrayed on one linkage group (termed "chromosome"), which may be transferred from cell to cell in an oriented and partial manner. Certain other genetic determinants (episomes) such as the sex factor may exist either attached or unattached to the primary linkage group and when unattached may be transferred independent of chromosomal transfer (2).

It is generally accepted that DNA is a primary carrier of genetic information in biological systems. Most of the DNA of *E. coli* has been shown to reside in a few

filamentous structures which appear to replicate semiconservatively (1, 4). It is tempting to identify such structures with the genetic linkage map and call them bacterial chromosomes.

The experiments reported here were designed to measure the amount of DNA transferred to single $F^-$ cells during bacterial conjugation and to follow the subsequent partition of radioactive label among the progeny of the $F^-$ recipients. Such experiments have been performed both for transfer of chromosome fragments (Hfr × $F^-$ matings) and for transfer of fertility factor ($F^+$ × $F^-$ matings). For comparison of the amounts transferred with the quantities of DNA found in the chromosome, we have tentatively identified the DNA structure which is conserved during vegetative replication (1, 3) with one-half of the bacterial chromosome. We have estimated by autoradiography DNA content of this conserved subunit for the strains and conditions used here in terms of "the average number of developed photographic grains per day of exposure." This is the same unit we have used for measuring amounts of label transferred during conjugation.

## MATERIALS AND METHODS

### *Strains*

All bacterial strains used derive originally from *E. coli* K12. The strains and their genotypes were the following.[1] HfrH (C. Levinthal): $Sm^s$; $F^+$ (T. F. Anderson): $Sm^s$, originally an Hfr but stocks inoculated from single colonies gave about $10^{-5}$ $T^+L^+Sm^r$ recombinants per input $F^+$ and high frequency transfer of F; $W_1F^-$ (C. Levinthal): $T^-L^-B_1^-Sm^r$; W2961F$^-$ (B. B. Beam): $T^-L^-B_1^-$ $Pro^-Sm^rLac^-$; WG2961F$^-$ (isolated from W2961F$^-$): $T^+L^+B_1^-Pro^-Sm^rLac^-$. We express our appreciation to those listed in parentheses for their generosity in supplying the strains indicated.

### *Media*

Liquid culture media were C minimal (5) supplemented with 0.2 per cent Difco vitamin-free casamino acids, 5 $\mu$g/ml of vitamin $B_1$ and 0.4 per cent glucose (designated CC medium), and Difco penassay broth. Minimal plates consisted of C minimal medium (with lactose substituted for glucose when desired) with 1.5 per cent Difco bacto-agar and supplemented, when needed, with 5 $\mu$g/ml vitamin $B_1$, 20 $\mu$g/ml threonine, 10 $\mu$g/ml leucine, 10 $\mu$g/ml proline, and 167 $\mu$g/ml streptomycin sulfate. Nutrient agar media for plates and coverslips contained 1.5 per cent and 1.0 per cent bacto-agar respectively, 0.8 per cent nutrient broth, and, as needed, 167 $\mu$g/ml streptomycin sulfate. Washing and diluting medium was C minimal without glucose.

### *Labeling of Cells*

Tritiated thymine (11.3 c/mM, New England Nuclear Corp., Boston) was purified chromatographically, as previously described for thymidine (3). The same specific activity was assured for all autoradiographic experiments to be compared quantitatively by

[1] Abbreviations: $Sm^s$, streptomycin-sensitive; $Sm^r$, streptomycin-resistant; T, threonine; L, leucine; $B_1$, vitamin $B_1$; Pro, proline; Lac, lactose; Ade, adenine; DNase, deoxyribonuclease; O = origin for chromosomal transfer; F, sex factor.

the use of a single lot of $H^3$-thymine as the sole source of thymine. Cells were labeled for about 5 generations, to titers not exceeding $5 \times 10^8$/ml in CC medium containing deoxyadenosine to promote the uptake of label (6). Control experiments showed that the amount of radioactivity incorporated per cell was independent of the concentration of deoxyadenosine in the range used (from 500 to 1000 $\mu$g/ml), the concentration of thymine (from 3 to 6 $\mu$g/ml), and the final concentration of cells (from $2 \times 10^8$ to $6 \times 10^8$/ml). DNase removed 98 per cent of the label from microcolonies initiated by labeled washed cells.

*Micromanipulation and Growth of Microcolonies*

Micromanipulation was performed as previously described (1). Growth of microcolonies was stopped (at about 16 to 32 cells per colony in the transfer experiments) by subjecting the cells to formaldehyde vapors for 4 minutes.

*Autoradiography*

The preparations were cytologically fixed and then autoradiographed as previously described (1), except that the following developing procedure at 20°C was used: 8 minutes in D-19 Kodak developer, 1/2 minute in $H_2O$, and 6 minutes in Kodak acid fixer followed by six 5 minute changes in $H_2O$.

Some preparations were treated with DNase (1x crystallized, Worthington Biochemical

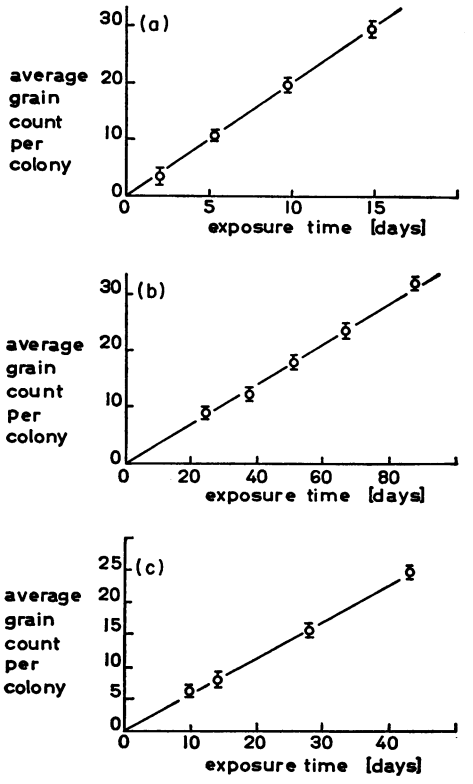



FIGURE 1 Grain counts as a function of exposure time over colonies initiated by washed, $H^3$-thymine–labeled cells. Each point represents the average grain count of at least 50 colonies. The error-bars represent one estimated standard deviation on each side of the average. Different specific activities were used in *a*, *b*, and *c* in order to examine different ranges of exposure time.

Corporation, Freehold, New Jersey, 100 $\mu$g/ml in 0.2 M maleate, 0.2 M tris, 0.01 M $MgSo_4$, pH 7.0) before they were autoradiographed. The buffer solution alone was used as a control. Warmed solutions were pipetted onto warmed coverslips and incubated at 37°C for 2 hours. The reaction was stopped by rinsing the samples in water and drying.

Fig. 1 shows that the response of the emulsion is linear over the range of exposures from 2 to 87 days. The reproducibility of the emulsions and the counting process was checked during the course of the work by comparing duplicate coverslips that were autoradiographed at different times. For 50 colonies scored on each coverslip, the standard deviation of the average was about 5 per cent. Out of a total of 6 comparisons made the largest discrepancy between any two duplicate coverslips was 13 per cent.

In the transfer experiments, average backgrounds varied between 0.3 to 0.6 grain per colony. Microcolonies with less than 4 grains were not tabulated in order to minimize the chances of counting colonies associated with only background grains. Background grains were distributed randomly, and the probability of a microcolony being associated with $\geq 4$ background grains was of the order of 0.001.

Grains appearing within a distance of about 1 $\mu$ of one another were scored as a cluster. This method of scoring permits one to characterize the degree of dispersion of the radioactivity among the progeny of the labeled cell initiating the colony. The response of the emulsion is roughly linear for clusters having an average grain count up to at least about 6 grains (see Fig. 9). Clustered grains are more difficult to count than grains that are well dispersed. It is estimated that for a cluster of about 7 grains, the tabulated count could often be in error by about 1 grain.

*Mating Procedures*

(*a*) *HfrH* × *W2961F*$^-$. After labeling, the Hfr strain was washed once in the cold and resuspended in CC medium containing 6 $\mu$g/ml of non-radioactive thymine and warmed to 37°C for about 2 minutes. It was then mixed with an aerated, log-phase culture of W2961F$^-$ grown in CC medium to give about 1 to 2 × $10^8$ cells per ml of each parent. Separation of mating couples was effected by sucking 1 ml of a chilled, diluted sample in and out of a Pasteur pipet for 1.5 minutes. Such interruption of mating will be referred to as "blendoring" since it was designed to accomplish the same purpose as the Waring blendor technique of Jacob and Wollman (2). Blendored samples were plated on minimal plates selective for recombinant types and on coverslips containing streptomycin–nutrient agar. A control experiment showed that a negligible quantity of unincorporated radioactivity remaining in the once-washed Hfr suspension was taken up by the F$^-$ cells.

The kinetics of recombinant formation (experiment BI) are shown in Fig. 2. After 1 to 2 hours of mating the number of T+L+Sm$^r$ recombinants was about 15 per cent of the input minority parent. No differences between these kinetics and those obtained in non-radioactive experiments have been observed.

(*b*) *F*$^+$ × *W*$_1$*F*$^-$. The labeled F$^+$Sm$^s$ strain was washed twice in the cold, resuspended in penassay broth, and mixed with an aerated, log-phase culture of W$_1$F$^-$Sm$^r$

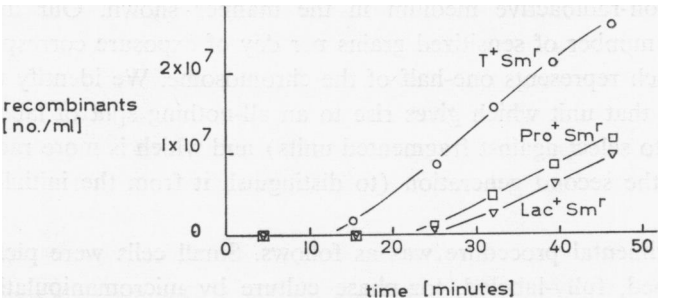



FIGURE 2 Kinetics of recombinant formation for radioactive experiment BI.

grown in penassay broth at 37°C to give about 1 to 2 × $10^8$ cells per ml of each parent. After chilling, diluting, and blendoring, samples were placed on plates and coverslips containing streptomycin–nutrient agar. Plates containing $Sm^r$ macrocolonies were stored at 4°C and later picked to test for fertility by mating with strain WG2961$F^-$. Fertility was judged by appearance of $T^+L^+Pro^+$ recombinants. When such recombinants appeared, they appeared at a frequency of about $10^{-8}$ per cent, which was at least 50 to 100 times higher than reversion to prototrophy of either parent.

## RESULTS

### 1. *Total Label per Chromosome*

We have examined the segregation of label among progeny of single, fully-labeled cells of the K12 strains used in the mating experiments. The patterns of segregation of label are the same as those reported for other *E. coli* strains (1), and the model previously presented for the organization and replication of DNA is used here. This model is depicted in Fig. 3 for young (short) cells of a log-phase popu-

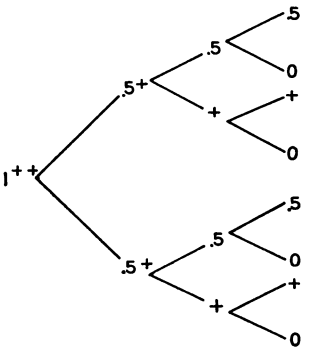


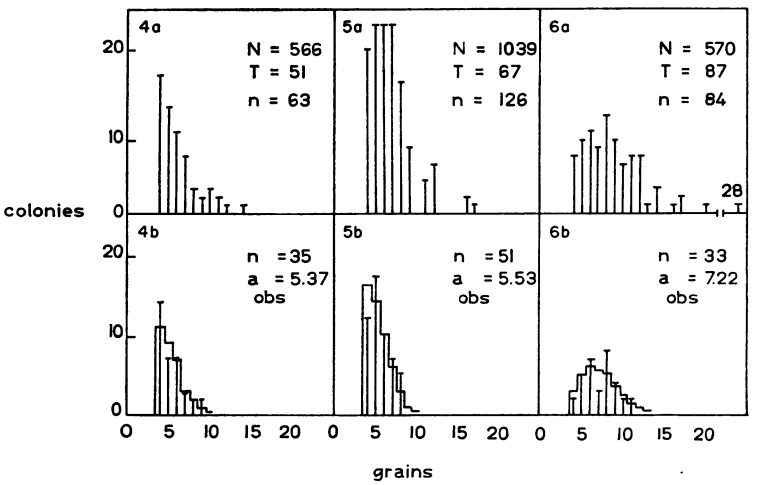

FIGURE 3 Schematic representation of the simplest distribution of labeled DNA among progeny of the fully-labeled young cells. The numbers represent relative amounts of labeled DNA contained by each cell, where $0 < + < 0.5$. The number 1 is meant to correspond to the DNA content of the bacterial chromosome.

lation. Such cells are interpreted as usually containing two completed subunits (each designated by 0.5 in the figure) and two unfinished subunits (designated by +). Barring fragmentation, these units, radioactively labeled, segregate among progeny

grown on non-radioactive medium in the manner shown. Our intention is to measure the number of sensitized grains per day of exposure corresponding to the 0.5-unit, which represents one-half of the chromosome. We identify the unit to be measured as that unit which gives rise to an all-nothing split of label at the third generation (to select against fragmented units) and which is more radioactive than its sister of the second generation (to distinguish it from the initially incomplete +-unit).

The experimental procedure was as follows. Small cells were picked out from a twice-washed, fully-labeled, log-phase culture by micromanipulation and their progeny isolated for at least 3 generations. The final isolates were allowed to form microcolonies (50 to 150 cells each) which were autoradiographed and grains from these colonies scored. The analysis discussed below was based only on clustered grains. About one-half of the non-clustered grains were due to background. The other half made up 12 ± 3 per cent of the total grains counted. Four experiments were performed, two each for HfrH and W2961F⁻. The results for the two strains were indistinguishable and are discussed together. All exposure times were close to 2 days.

Two hundred eight cell divisions were observed at the third generation. Of these, 74 gave rise to all-nothing splits and 36 gave all-nothing splits which con-




FIGURES 4 to 6 Grain count distributions of experiment FI. Symbols: $N$, total number of $Sm^r$ colonies scanned; $T$, exposure times in days; $n$, number of labeled colonies represented in histogram; $a_{obs}$, observed average grain count. For a Poisson distribution the average grain count, $\bar{a}$, is obviously smaller than the average obtained if grain counts less than 4 are not included, $a_{obs}$. The relation between $a_{obs}$ and $\bar{a}$ may be represented graphically (7). Each of the expected Poisson distributions drawn in Figs. 4*b* to 6*b* was obtained uniquely from $a_{obs}$ by first determining the corresponding value of $\bar{a}$.

tained more developed grains than their sisters of the second generation. The average grain count of the latter 36 was 10.7 ± 0.5 grains per 2-day exposure. This would be the measurement we are interested in except for four sources of error.

The first concerns the fact that even though the 0.5-unit will emit more electrons per unit time on the average than the +-unit, it will not emit more electrons during every particular interval of time. This means we might have overestimated the size of the 0.5-unit because we might have omitted some of the lower tail of the Poisson distribution in the averaging process. Each of the other errors leads to an underestimate of the size of the chromosome. One involves the effects of saturation of the emulsion due to clustering of photographic grains. Another concerns the possibility that the 0.5-unit could fragment before the third generation and still give rise to an all-nothing split at the third generation without being as radioactive as it would have been without the fragmentation. The final source or error concerns the nonclustered grains, since it is not known what fraction of these were due to label contained in the chromosome.

From detailed evaluation of the grain-count data for the maximal effects of each of these sources of error we have concluded that 9 and 15 grains per day of exposure represent respectively the lower and upper bound for the size of the chromosome. This amounts to 25 to 45 per cent of the average label per cell for log-phase cultures of HfrH and F+.

2. *Transfer of Label During F+ × F− Matings*

(*a*) *Distributions of Total Grain Counts per Colony.* In Figs. 4*a* to 7*a* are presented the results obtained on numbers of $Sm^r$ microcolonies associated with different numbers of total developed grains. The data were obtained from two experiments, designated "FI" and "FII." The mating times were 60 minutes for FI and

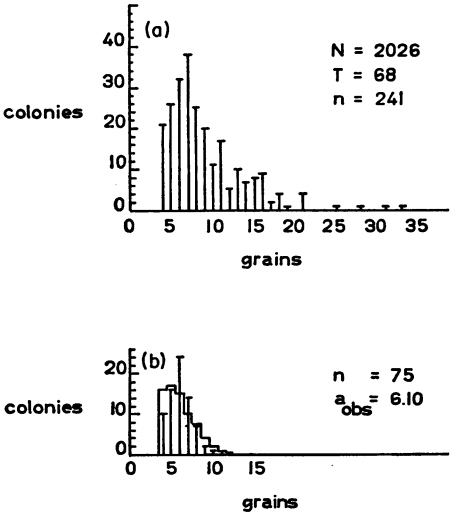



FIGURE 7 Grain count distributions of experiment FII. For explanation and symbols see caption to Figs. 4 to 6.

25 minutes for FII. The 85 to 89 per cent of the colonies which did not contain at least 4 grains had an average total grain count (about 0.7 grain per colony) not very different from background (about 0.5 grain per colony). After DNase treatment the grain counts over all $Sm^r$ colonies were near the background level.

Table I shows that the fraction of $Sm^r$ colonies that received the sex factor is close to the fraction that is labeled. The fractions labeled have been corrected for the estimated number of colonies with less than 4 grains which contained as much

TABLE I

F TRANSFER AND LABEL TRANSFER

| Experiment | No. of $Sm^r$ colonies checked for F | No. of $Sm^r$ colonies with F | $Sm^r$ colonies labeled |
|---|---|---|---|
| | | | *per cent* |
| FI | 7 | 3 | 16 |
| FII | 60 | 8 | 15 |
| FIIS | 67 | 0 | <1 |

radioactivity as the conserved unit discussed below. Experiment FIIS was the same as FII except that the mating mixture contained 200 $\mu$g/ml of streptomycin sulfate. Streptomycin was added in order to inhibit DNA synthesis during mating, but no transfer of F was detected. The experiment thus serves as a control.

The distributions of Figs. 4*a* to 7*a* are broader than expected for a population of colonies containing the same amount of transferred label and thus do not give good fits to Poisson distributions. The significance of this fact, however, should be considered in relation to the segregation of the label among exconjugant progeny to be described next.

(*b*) *The Conserved Unit.* A number of labeled microcolonies were found for which all of the developed grains over each colony appeared in one cluster. One such colony is pictured in Fig. 8. In Figs. 4*b* to 7*b* are given the grain count distributions for colonies in which all of the radioactivity appeared in one cluster. Also included are the clusters from colonies in which all but 1 grain over the colony appeared in one cluster. Most of these single grains may be attributed to background. Inspection of the graphs indicates that the data are not very different from Poisson distributions. The $X^2$ test for comparison of observed and expected distributions gives $P > 0.75$, $> 0.5$, $> 0.5$, and $> 0.01$ for Figs. 4*b* to 7*b* respectively. These values are judged to represent reasonable agreement between the experimental and Poisson distributions, considering the recognized difficulties in counting clustered grains. In Fig. 9 are plotted the average grain counts of the conserved unit (corrected for the fact that grain counts of 4 grains were not tabulated) *versus* exposure time. From this graph it is estimated that the radioactive unit that is conserved gave rise

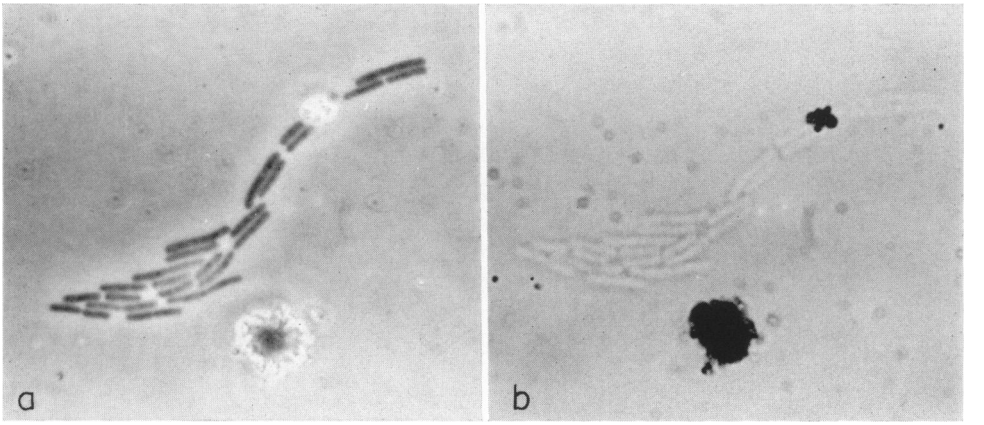



FIGURE 8 Autoradiogram showing a colony containing one cluster of radioactivity. Also shown is a heavy concentration of developed grains due to presence of a radioactive $F^+Sm^r$ cell. (*a*) phase contrast microscopy, (*b*) bright field. Exposure time: 87 days. × 2000.

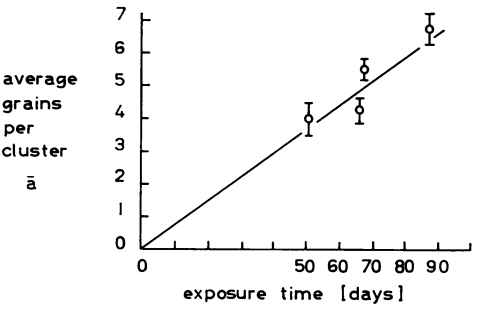



FIGURE 9 Average grains per cluster, $\bar{a}$, as a function of exposure time for one-spot colonies. See caption to Figs. 4 to 6 for manner of relating $\bar{a}$ to $a_{obs}$.

to 0.077 ± 0.007 grain per day of exposure on the average. This amounts to 0.5 to 0.9 per cent of the chromosome, where the upper and lower extremes were determined from the lower and upper extremes for the size of the chromosome respectively.

Each of the distributions of Figs. 4*b* to 7*b* was taken from the populations whose total grain counts are given in the corresponding figures (4*a* to 7*a*). A comparison of the figures indicates that a large fraction of the labeled population contains amounts of label corresponding to the size of the conserved unit. The broadening of the total population distributions to the high side of the distributions of Figs. 4*b* to 7*b* can possibly be accounted for by the following considerations. First, no background corrections have been made on the total populations. Secondly, if the label in single-cluster clones corresponds to a single sex factor or a subunit of it, one

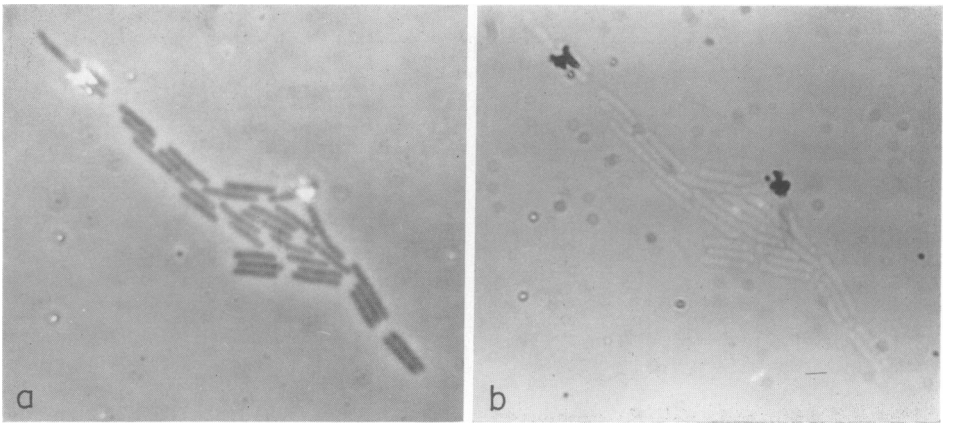



FIGURE 10 Autoradiogram of a two-spot colony. (*a*) phase contrast, (*b*) bright field. Exposure time: 68 days. × 2000.

might expect some $F^-$ cells to receive more than one sex factor or labeled subunit. Suggestive of this are a few colonies containing about twice the radioactivity of the single-cluster colonies and localized into two clusters of about equal size. One such two-spot colony is pictured in Fig. 10. Thirdly, at some frequency there will be chromosomal transfer which will, in general, contribute relatively high grain counts.

(*c*) *Dispersion of Grains Over Radioactive Colonies.* The radioactivity in the labeled colonies did not always appear in pieces the size of the conserved unit. The average number of grains per cluster (corrected for background) for the distributions of Figs. 4*a* to 7*a* were 3.3, 3.0, 3.7, and 3.4 respectively. These values are all appreciably smaller than the average grain counts obtained for the conserved unit (4.0, 4.3, 6.7, 5.2 for Figs. 4*b* to 7*b* respectively). This dispersion of radioactivity over labeled colonies could be due to fragmentation of the sex factor during growth of the colony, similar to fragmentation of labeled units already shown to occur during chromosomal replication (1). However, the possibility that the dispersal of grains is due to units (sex factor or other) containing less radioactivity than the conserved unit and which segregate out during growth of the recipient colony, cannot be ruled out.

3. *Transfer of Label During Hfr × $F^-$ Matings*

(*a*) *Distributions of Total Grain Counts per Colony.* In Figs. 11 and 12 are presented total grain count distributions for $Sm^r$ microcolonies initiated by females which were mated with radioactive males for different periods of time. Two experiments, BI and BII, are represented. Each point (marked by a dot—the squares are discussed below) gives the number of $Sm^r$ colonies with $\geq x$ grains per day of exposure. The small grain counts tabulated for the 3 minute and 2 minute samples do not necessarily represent transferred radioactivity. Uptake of residual radioactivity left in the mating medium together with background grains could account for the

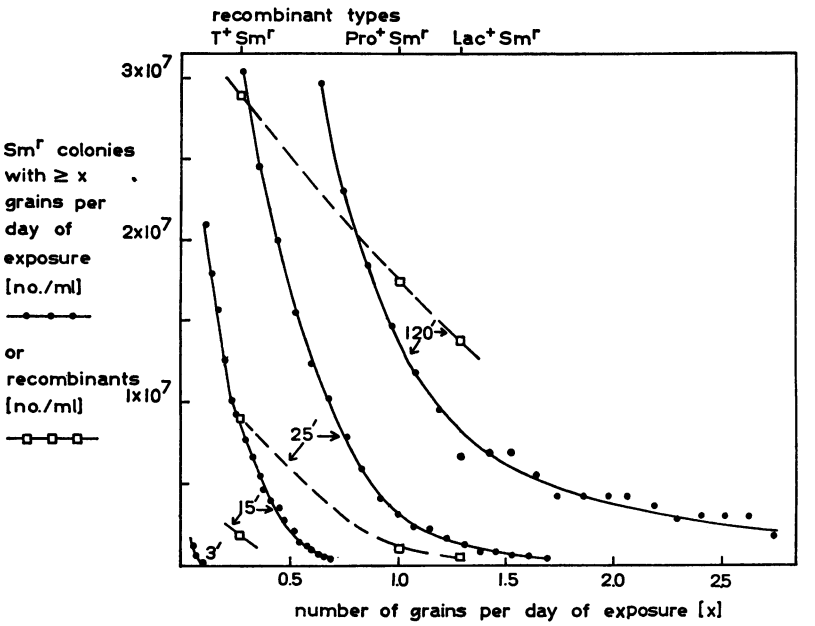



FIGURE 11 Grain count distributions of experiment BI. The numbers of colonies are given per milliliter of mating mixture. These values were determined from the ratio of labeled Sm$^r$ colonies to total Sm$^r$ colonies scanned and from the total number of cells per milliliter of the mating mixture (sampled at the appropriate time) which gave rise to Sm$^r$ colonies on Sm nutrient agar plates.

| Sampling time | Exposure | Total No. of Sm$^r$ colonies scanned | No. of labeled colonies counted |
|---|---|---|---|
| *min.* | *days* | | |
| 3 | 59.3 | 1000 | 7 |
| 15 | 32.2 | 2981 | 216 |
| 25 | 12.8 | 4393 | 348 |
| 120 | 9.0 | 1403 | 64 |

resulting distributions. The very small degree of labeling of these colonies indicates, however, that the colonies from the later samples did not receive their label during any of the experimental manipulations after the samples were taken but rather received it while in the mating mixture. DNase treatment of a 120 minute sample reduced the grain counts over Sm$^r$ colonies to background levels.

Qualitatively, for each experiment the distributions are shifted toward higher grain counts with increasing time. This result implies that individual Sm$^r$ cells in the mating mixture receive progressively more label with time; that is, the total increase is not simple due to an increased number of recipients containing similar amounts

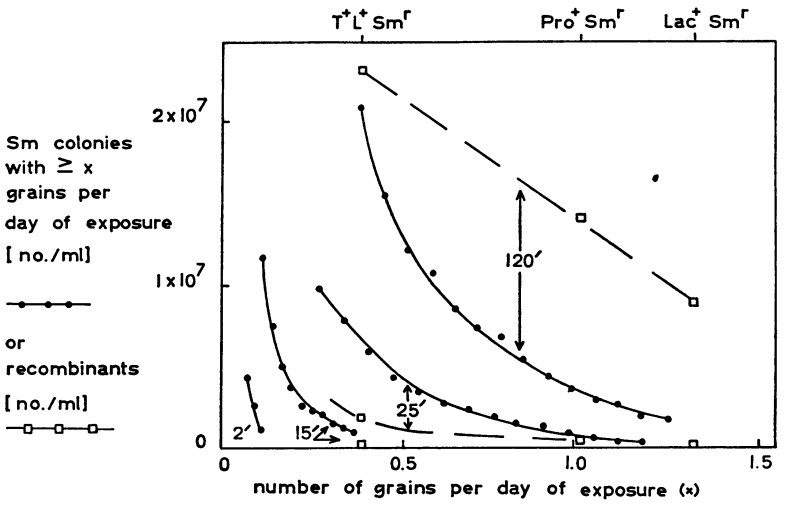



FIGURE 12 Grain count distributions of experiment BII.

| Sampling time | Exposure | Total No. of Sm$^r$ colonies scanned | No. of labeled colonies counted |
|---|---|---|---|
| *min.* | *days* | | |
| 2 | 53.7 | 500 | 25 |
| 15 | 34.3 | 1020 | 98 |
| 25 | 14.1 | 2908 | 176 |
| 120 | 15.0 | 1500 | 70 |

of transferred DNA. This provides direct evidence that blendoring does rupture the genetic material while it is in the process of being transferred or that it at least leads to early loss of material from the zygote and that it does not simply render the injected chromosome non-functional beyond some point along its length.

The distributions of label also show that the Sm$^r$ colonies were extremely non-uniformly labeled as expected on the basis of genetic experiments (2). The colonies that were associated with $\geq 4$ grains gave much broader distributions than Poisson distributions. For qualitative comparison with the grain count distributions, the numbers of recombinants per milliliter corresponding to different times of blendoring are also plotted (the squares) in Figs. 11 and 12. The abscissae for these data were determined in the following manner. The total genetic map of *E. coli* corresponds to about 108 minutes of chromosomal transfer (2). The fraction of chromosome corresponding to the distance from the origin to a given marker can be determined from the time of entry of the marker with one additional consideration. This is that the distance is smaller than that estimated from the time of entry directly, probably because injection does not begin immediately after mixing the parents. For ex-

ample, the marker $Ade_{13}$, which lies on the distal end of the HfrH chromosome, has been shown to be about 4 minutes from T on other Hfr's (8). Therefore, the segment O–T in HfrH must be less than 4 minutes. The distance O–T must also be at least 2 to 3 minutes since one of the determinants for fimbriation has been located 2 to 3 minutes on the proximal side of T in HfrH (9). Thus, the distances O–T, O–L, O–Pro, and O–Lac for HfrH are estimated to correspond to about 3, 4, 10, and 13 minutes of transfer respectively. The corresponding fractions of the chromosome are 3/108, 4/108, 10/108, and 13/108. The grain count coordinates for plotting the numbers of recombinants in Figs. 11 and 12 were obtained by multiplying these fractions by our estimate for the size of the chromosome, 11 grains per day of exposure. With the definition of the coefficient of integration, $c$, as the fraction of zygotes receiving a chromosomal length O–A or greater which becomes recombinant for marker A, the 15 minute and 25 minute grain count curves in Figs. 11 and 12 show an approximate correspondence with the recombinant curves for $c$ in the range 0.2 to 0.6.

The 120 minute grain count curves do not fit the recombinant curves, however, since the values of $c$ would in some cases be much larger than 1. This is not surprising for two reasons. First, it may be anticipated that considerable unlabeled DNA, synthesized by males while in the non-radioactive mating medium during the long period of mating, may be transferred. Secondly, recipient cells may segregate transferred radioactivity by cell division in the mating medium, in which case the radioactivity of a transferred fragment would appear in more than one $Sm^r$ colony. For both of these reasons one might expect a lower average grain count per labeled colony than that predicted by the model, and this is what is observed. Indeed, we infer from the results that one or both of the events described above has occurred in the case of the 120 minute samples.

The purpose of the rest of this section is to calculate from the grain count data of the 15 minute and 25 minute samples quantitative limits for the amounts of transferred radioactivity corresponding to the distances O–T, O–TL, and O–Pro. Since an independent measure of the coefficient of integration has not been made in this work, we shall present the results calculated for values of $c = 0.25$ and 0.50. These values are consistent with the comparisons discussed above for Figs. 11 and 12. They are also the values obtained by the method of zygotic induction by de Haan and Gross (10) and Jacob and Wollman (11), respectively, although it is recognized that $c$ can depend on strains and media used. It is noted that we expect $c$ to be independent of the length of the fragment beyond O–A and therefore independent of the blendoring time (10). We also expect that $c$ will be approximately constant for selected markers that are located near one another on the linkage map (11).

The calculations were performed as follows. The fraction of $Sm^r$ colonies which is recombinant for A is known. The amount of radioactivity corresponding to the

distance O–A is found by locating the values of grain count, $\alpha$, such that the fraction of $Sm^r$ colonies with $\geq\alpha$ grains divided into the recombinant fraction is equal to $c$ within the estimated limits of experimental error for each experiment.

The errors involved in this calculation are the following: a statistical error in measuring the fraction of $Sm^r$ colonies with $\geq x$ grains; statistical and pipetting errors in measuring the fraction of recombinants; and a systematic error due to the fact that the expected number of colonies with $\geq\alpha$ grains is slightly higher than the number of colonies containing $\geq$ the amount of radioactivity that gives $\alpha$ grains on the average.[2]

The ranges of grain counts consistent with these estimated uncertainties for the marker distances O–T, O–TL, and O–Pro have been calculated for the 15 minute and 25 minute grain count distributions of experiments BI and BII. In order to compare the calculated amounts of transferred label with the size of the chromosome, each lower limit was divided by 15 grains per day of exposure (upper estimate for size of chromosome) to obtain a lower limit on the per cent of the chromosome represented by the marker distance. Similarly, each upper limit was divided by 9 grains per day of exposure. This method of compounding errors was used because systematic errors were involved in setting maximal limits on the size of the chromosome. The resulting values are given in Table II and are to be compared with the estimated fractions of the genetic map for O–T, O–TL, and O–Pro of roughly 3, 4, and 9 per cent respectively. Where blanks occur in the table, no limits could be set. Calculations for the segment O–Lac were characterized by much wider ranges of possible values than the segments given and are therefore not included. The relative amounts of transferred radioactivity agree within the relatively large experimental errors with the genetically measured distances.

Finally, it should be pointed out that the 25 minute curves of Figs. 11 and 12 are not expected to be simply displaced by 10 minutes of transfer toward higher grain counts from the 15 minute curves. This is because many (roughly one-half, based on the fact that after 120 minutes of mating the ratio of $Lac^+Sm^r$ recombinants to

[2] If $f(x)$ = the fraction of colonies containing $\geq$ the amount of radioactivity that gives rise to $x$ grains on the average, and $P(n)$ = the fraction of colonies containing $\geq n$ grains, then $f(x)$ and $P(n)$ are related by the following formula:

$$P(n) = \sum_{s=n}^{\infty} \int_0^{\infty} \left(-\frac{df}{dx}\right) \frac{x^s e^{-x}}{s!}\, dx$$

Thus, for a given $f(x)$ one may calculate $P(n)$. This has been done numerically for curves of expected shape for $f(x)$. It is found that the resulting $P(n)$ closely approximates $f(x)$ $(n = x)$ with the biggest discrepancy occurring at the upper tail of the distribution where $P(n)$ is slightly larger than $f(x)$. Over the greater part of each curve, this is a minor error compared to the other inaccuracies. However, the "smoothing out" of the upper tail of the grain count distribution makes a calculation of the amount of radioactivity corresponding to the largest (*i.e.* earliest injected) chromosomes transferred after particular periods of mating virtually impossible.

TABLE II

TRANSFERRED MARKER DISTANCES CALCULATED FROM ASSUMED VALUES FOR COEFFICIENT OF INTEGRATION

| Marker segment | Sample | Per cent of chromosome | | Per cent of genetic map |
|---|---|---|---|---|
| | | $c = 0.25$ | $c = 0.50$ | |
| O–T | 15 min. BI | 1.7 to 4 | 2.5 to 6 | ~3 |
| O–T | 25 min. BI | —4 | 2.6 to 6 | ~3 |
| O–TL | 15 min. BII | 2.3— | — | ~4 |
| O–TL | 25 min. BII | —5 | 3 to 7 | ~4 |
| O–Pro | 25 min. BI | 5 to 11 | 6 to 14 | ~9 |
| O–Pro | 25 min. BII | 4 to 10 | 5 to 12 | ~9 |

$T^+Sm^r$ recombinants is about 0.5) of the chromosomes being injected rupture spontaneously during 10 minutes of transfer so that the increase in amount transferred is not the same for all recipients and is less, on the average, than 10 minutes of transfer. (In addition, it has been shown (10) that some HfrH donor cells inject at slower rates than others.)

(*b*) *Dispersion of Grains Over Radioactive Colonies.* Radioactivity transferred by HfrH donors tended to be more dispersed over recipient colonies than that transferred by $F^+$ donors. As an indication of this dispersal, for the class of colonies associated with at least 10 grains (from the 15 minute and 25 minute points of experiment BI) the average number of grains per cluster was approximately 2.

No attempts were made to study segregation of label in exconjugant pedigrees by micromanipulation. It was, nevertheless, possible to read some pedigrees from labeled small colonies blendored at 25 minutes and autoradiographed for longer times than experiments BI and BII. Although no single pattern emerged, several examples of clones were found in which a large fraction of the label persisted in one subline for 4 to 5 generations giving rise to sublines with only small amounts of label.

From micromanipulation studies on the segregation and recombination of genetic traits among the progeny of $F^-$ exconjugants, Anderson (12) has proposed that the fragment of an Hfr chromosome may persist in a single line descended from the $F^-$ zygote for many divisions and during this time it may recombine a number of times with the $F^-$ genome. Our results indicate the injected DNA does not, in general, persist intact for the strains and conditions employed in these experiments, although it is not impossible that some fraction of it does.

(*c*) *Transfer from $F^-$ to Hfr.* As a control on the specificity and directionality of transfer, an experiment was performed in which labeled $F^-$ were mated with unlabeled Hfr. Recombinants were also measured on these mating cultures.

Fig. 13 shows the results of this experiment, in comparison to experiment BI. Female colonies contained large amounts of label and thus could be distinguished from males. Surprisingly, some male clones did contain measurable label, although much less than that transmitted to females when males were labeled. The heterogeneity in amounts of label per colony argues for some relation to the mating process. No further attempts to study this reverse transfer were made. For comparison, it should be noted that Garen and Skaar detected a small transfer from female to male in their studies using $P^{32}$ (13).

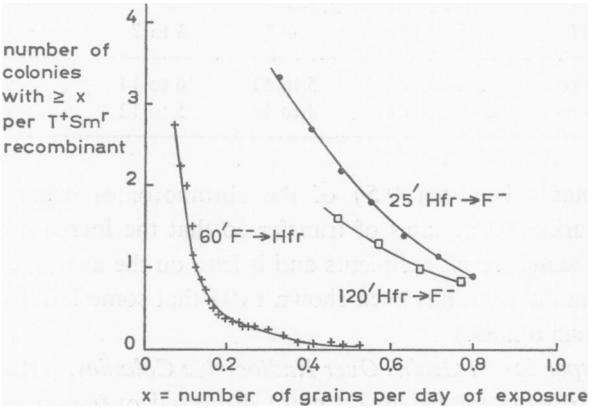



FIGURE 13 Transfer of label from $F^-$ to Hfr.

## DISCUSSION

The most striking feature of the transfer experiments was the high frequency at which the label transferred by an F+ donor remained intact during growth of the recipient colony. The value of 0.5 to 0.9 per cent of the chromosome for the size of the conserved unit is to be compared with the value of 1 to 2 per cent of the chromosome for the DNA content of the sex factor as determined by Driskell-Zamenhof and Adelberg by $P^{32}$ suicide (14). The closeness of these two values, together with our correlation between F transfer and label transfer, indicates that the DNA of the conserved unit is a large part of the DNA of the sex factor.

Analyses of the descendents of zygotes formed in F+ × F− matings have shown that the sex factor is inherited by all descendents of a zygote (15, 16). This implies that the label is not conserved simply because there is no replication of the sex factor. A likely interpretation of the results is that the DNA of the sex factor replicates semiconservatively prior to transfer while the donor is in the non-radioactive mating medium. The transferred unit then contains one radioactive subunit and one non-radioactive subunit, each of which tends to remain intact during further semiconservative replications. With this interpretation it is somewhat surprising that

so many one-spot colonies occurred for a mating time of only 25 minutes (generation time of F+ in CC medium was about 45 minutes). Possibly the transfer of F–DNA may be related to its synthesis in some way that guarantees the transfer of one old (labeled) and one new (unlabeled) subunit. Some clarification of this issue might be obtained by performing the experiment under conditions such that either the mating takes place in the absence of DNA synthesis or only the males are able to incorporate radioactive thymine from the mating mixture. One might then expect to see at least two conserved units in each recipient colony.

No matter what explanation is invoked to explain the one-spot colonies, it is likely that they have received only one labeled sex factor. The transfer of close to one sex factor per mating pair is consistent with the one-hit $P^{32}$ suicide kinetics (14, 17).

In considering the process of chromosomal transfer by Hfr's, one wonders what physical relation there is between the fragment that is transferred and the chromosome structure for which it originated. For example, we could imagine an Hfr transferring a linear structure containing anywhere from one to four or possibly more strands of DNA. Judging from the amounts of label corresponding to particular marker distances, our results favor the transfer of two labeled strands for short mating times, although the possibility that only one labeled strand is transferred cannot be excluded. The transfer of four labeled strands is very unlikely.

Indirect evidence consistent with the hypothesis that the chromosome is a long DNA backbone which is transferred in a linear, oriented manner by Hfr donors, was first provided by Fuerst, Jacob, and Wollman (18). These workers followed the inactivation of particular recombinations by $P^{32}$ decay in HfrH prior to mating. Experiments by Marcovitch, similar in design but using x-irradiation of males instead of $P^{32}$ decay, have given results completely analogous with those obtained by $P^{32}$ suicide (19). These kinds of experiments give poor absolute measures of amounts of material transferred, however. Direct measurements on amounts of DNA transferred during mating, all of which involved measuring bulk transfer from one cell type to another, have been performed in several laboratories (13, 20, 21, 22). Insofar as comparisons can be made, our results are in agreement with the measurements of others on amounts of transfer by Hfr donors. Since relatively long periods of mating were used in these earlier experiments, one wonders how much unlabeled DNA synthesized by the donors while in the nonradioactive mating mixture was transferred. Furthermore, in order to compare measurements on bulk transfer with those expected from a genetic model, it is necessary to make some estimate of the average amount of genetic material transferred per mating event. This estimate is complicated by the fact that each injected chromosome is subject to spontaneous rupture while it is being transferred. The heterogeneous distributions of transferred chromosomal lengths expected as a result of this breakage phenomenon were observed in the experiments reported

here. No assumptions concerning the degree of breakage were involved in our comparisons of experimental and predicted amounts of label transferred. In common with previous work, however, our estimates for the amounts of transferred DNA corresponding to particular marker distances depended on assumed values for the coefficients of integration. One can see from Table II that the lower the coefficient of integration, the less the measured amount of transferred chromosome per marker distance.

Bouck and Adelberg (23) have suggested that initiation of transfer is a property of chromosomes whose replication has been recently completed but not of those in the process of replication. This model predicts decreasing amounts of label transferred for a given length of chromosome transferred as mating proceeds, in experiments such as reported here. Short mating times would, however, yield close to two-strand amounts of transferred label, particularly for the larger fragments which represent early injection. The errors inherent in our measurements of the amount of transfer would not admit a decision on small differences from the two-strand quantity.

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