Deoxyribonucleic Acid Transferred from Ultraviolet-Irradiated Excision-Defective Hfr Cells of Escherichia coli K-12

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Deoxyribonucleic acid (DNA) transfer from 3H-thymine-labeled Hfr cells has been measured by determining the amount of radioactivity remaining after selective lysis of the donor cells in the mating mixture. DNA transfer was less effectively reduced by ultraviolet irradiation of excision-defective Hfr cells than was the yield of recombinants. The buoyant density of DNA transferred from unirradiated and irradiated Hfr cells was equivalent to that of double-stranded DNA. Mating-dependent DNA synthesis in the recipient has been measured by mating Hfr cells deficient in thymidine kinase with irradiated thymine-requiring F' cells in the presence of 3H-thymine. The extent of such DNA synthesis approximated the amount of DNA transferred from unirradiated donors. Neither DNA transfer nor mating-dependent DNA synthesis could be reliably measured when both parents were irradiated. It is proposed that transferred Hfr DNA is replicated in the recipient and that this replication still occurs when the Hfr DNA contains dimers.

The principal photoproducts induced in deoxyribonucleic acid (DNA) by ultraviolet (UV) irradiation are dimers between adjacent pyrimidine bases. Strains of Escherichia coli which carry a mutation at one of the uvr loci are abnormally sensitive to UV and are unable to repair the UV-damaged DNA by the excision process (see 6 for review). Excision-defective strains which are also mutant at recA (a locus for recombination-proficiency) are considerably more sensitive to UV than either of the single mutants, which suggests that the lethal effects of UV can be reduced by a second process which involves genetic recombination (9).

Repair by recombination is thought to occur after the replication of dimer-containing DNA, when the newly synthesized strand is discontinuous and has gaps opposite the dimers in the template strand (8, 19). The discontinuous strand of DNA is subsequently converted into material of normal molecular weight. This conversion, which does not take place in recA cells (21, 22), is thought to involve genetic exchanges between the defective regions and homologous, intact DNA (8, 19).

UV-induced recombination has been studied in E. coli K-12 mating systems in which excision-defective Hfr or F' donor cells were irradiated before mating (8, 23, 27). Irradiation of the male cells results in the incorporation of small segments of the exogenote into the recipient DNA, and this effect is not influenced by the capacity of the female cell to excise dimers. The explanation put forward to account for these genetic data is that the exogenote is composed of a dimer-containing strand from the donor and a discontinuous daughter strand and that some feature of the dimer-gap combination stimulates genetic exchanges.

The proposal is consistent with a model of conjugation in which the donor DNA is replicated during transfer (11). More recent work on bacterial conjugation has shown that only a unique strand of DNA formed in the donor can be recovered from the recipient (16, 20; J. A. Fralick and W. D. Fisher, Bacteriol. Proc., p. 54, 1970). This implies that, if there is replication of transferred DNA during mating, it occurs in the F'. A precedent for this suggestion may be found in the work of Ptashne (18) and Gross and Caro (4).

A second hypothesis (23) to explain the ge-
nomic data obtained by mating irradiated Hfr donors is that the normal replication of the transferred DNA is prevented by the dimers, and the altered patterns of recombination result from the exogenate being single-stranded. That a single-stranded exogenate is derived from unirradiated Hfr donors has been proposed by Kunicki-Goldfinger (12) and Curtiss (2).

The purpose of the experiments reported here was to investigate whether the DNA transferred during mating from UV-irradiated, excision-defective Hfr cells is single- or double-stranded in the F² cells. To do this, we examined the buoyant density of transferred DNA isolated from recipient cells after mating. We also used a mating system, in which a thymine-requiring recipient is heavily irradiated and the Hfr is unable to incorporate thymidine (3), for measuring DNA synthesis in the recipient after transfer from an Hfr donor.

MATERIALS AND METHODS

Bacterial strains. The strains of E. coli K-12 used are described in Table 1. BW40 and BW119 were derived by PI cotransduction of the tkd-I mutation from KY895 (5, 10) into trp mutants isolated, after N-methyl-N-nitroso-N'-nitroguanidine mutagenesis, from AB1885 and AB3108 thy², respectively. BW26 was obtained by the triumphetorim selection method (24).

Media. G medium contains per liter: Na₂HPO₄, 6 g; KH₂PO₄, 3 g; NaCl, 0.5 g; NH₄Cl, 1 g; CaCl₂, 11 mg; MgSO₄·7H₂O, 0.25 g; Difco Casamino Acids, 10 g; thiamine hydrochloride, 1 mg; glycerol, 10 ml. Buffer contains per liter: KH₂PO₄, 3 g; Na₂HPO₄, 7 g; NaCl, 4 g; MgSO₄·7H₂O, 0.1 g; pH 6.9.

Growth. Cells were grown overnight in G medium supplemented with 10 μg of thymine per ml for thymine-requiring strains. The overnight culture was diluted in G medium containing 2 μg of thymine per ml for thymine-requiring strains, and aerated at 37°C for about three generations to approximately 2 × 10⁸ cells/ml. The generation time was about 47 min.

Irradiation. Cell suspensions (10-ml) were irradiated in 9-cm diameter petri dishes at 254 nm with a germicidal lamp. Dose rates were adjusted by using a dosimeter constructed by R. Latarjet (13). Hfr cells were irradiated after a fivefold dilution in buffer. F² cells were irradiated without dilution.

Mating. Unless otherwise specified, the mating mixtures contained, per ml, 5 × 10⁸ to 7 × 10⁹ Hfr cells and approximately 2.0 × 10⁸ recipient cells and were supplemented with 100 μg of thymidine per ml. Mating mixtures of 3.5 ml were shaken gently at 37°C in 150-ml Erlenmeyer flasks. Pro⁻ Thy⁺ T6⁺ recombinants were assayed by blending a sample of the mating mixture for 10 sec (14), incubating the cells with phage T6 for 15 min, and plating the suspension on an appropriate selective medium.

Removal of donor DNA from the mating mixture. The method for removal of donor DNA from the mating mixture was based on those of Matsubara (15) and Ohki and Tomizawa (16). This depends on sensitivity of the Hfr to T6 bacteriophage. A 2.5-ml amount of mated mixture was added to 0.2 ml of 0.175 M KCN. This was blended for 10 sec before addition to 0.5 ml of T6 containing 5 × 10¹⁵ particles, UV-irradiated with 800 ergs/mm². Deoxyribonuclease (DNase, Calbiochem B) and ribonuclease (RNase, Calbiochem A) were added to final concentrations of 150 and 100 μg/ml, respectively. The mixture was held at 37°C for 15 min. Pronase (Calbiochem B), predigested for 60 min, was added to give a concentration of 375 μg/ml. After a further 15 min of incubation, Brj 58 (Honeywell Atlas) was added to 0.5%, and the mixture was cooled to 0°C. After 5 min, the cells were centrifuged and washed three times in buffer containing 100 μg of thymine per ml. The pellets were resuspended in 2.5 ml of water for assay of the remaining thchloroacetic acid-precipitable ¹H or in 1 ml of SET [10⁻⁸ M tris-(hydroxymethyl)aminomethane (Tris), 10⁻² M ethylene-diaminetetraacetic acid (EDTA), 10⁻¹ M NaCl, pH 8.1] for lysis of the recipient cells.

Lysis of recipient cells. Lysozyme was added to the cells in SET to a final concentration of 500 μg/ml. After three cycles of rapid freezing and thawing, Sarkosyl NL97 (Geigy, U.K.) was added to 0.4%. The mixture was held at 0°C for about 5 min. Pronase (predigested for 20 min) was added to 470 μg/ml, and digestion was continued at 37°C for 2.5 hr. The lysate was blended in a Vortex mixer for 30 sec to fragment the DNA.

Density-gradient equilibrium centrifugation. To 6.5 g of CsCl (Merck suprapur) were added 1.4 ml of lysate, 3.5 ml of 0.3 M K₂HPO₄ (pH 10.9), and 0.1 ml and 0.23 ml of native and denatured ¹H-reference DNA, respectively. The pH was adjusted to 11.0, and the den-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>thyA</th>
<th>drm</th>
<th>tkd</th>
<th>uvr</th>
<th>pro</th>
<th>lac</th>
<th>T6</th>
<th>Derived from</th>
</tr>
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<tbody>
<tr>
<td>AB1885</td>
<td>F⁻</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>BW40</td>
<td>F⁻</td>
<td>+</td>
<td>+</td>
<td>-1</td>
<td></td>
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<td></td>
<td>AB1885</td>
</tr>
<tr>
<td>BW140</td>
<td>F⁻</td>
<td>+</td>
<td>+</td>
<td>-1</td>
<td></td>
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</tr>
<tr>
<td>BW26</td>
<td>F⁻</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BW126</td>
<td>F⁻</td>
<td>-</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>AB3108</td>
<td>HfrJ</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>BW119</td>
<td>HfrJ</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AB3108</td>
</tr>
</tbody>
</table>

⁠aThe symbols for genotype are those used by Taylor (25). Cells mutant at thyA and drm (structural genes for thymidylate synthetase and deoxyribonuclease) can grow in low concentrations of thymine.
sity of the solution was adjusted to 1.72 g/cm². A 6.5-
ml amount was added to cellulose nitrate tubes, pre-
treated by boiling in 10⁻³ M EDTA (20), and centri-
fuged for 38 to 42 hr at 35,000 rev/min at 15 °C, in a
no. 65 rotor in a Spinco L2 centrifuge. The radioac-
tivity in 10-drop fractions was assayed on 3MM discs
by method A (see below).

³²P-reference DNA. ³²P-DNA was prepared from
AB3108 grown in medium containing 40 µg of P and
20 µCl of ³²P per ml. The cells were lysed with lyso-
zyme and Sarkosyl. The DNA was purified by two equili-
rium centrifugations in CsCl, followed by di-
alysis against 10⁻³ M Tris, 10⁻³ M EDTA, pH 8.0. The
DNA was denatured by raising the pH to 12.5 with
KOH followed by incubation for 10 min at 37 °C. The
solution was then neutralized with HCl.

Estimation of radioactivity. Method A: Trichloro-
acetic acid-precipitable radioactivity was measured in
triplicate samples collected on 2.5-cm 3MM discs
(Whatman), which had been treated with 1 n NaOH.
These were washed by the procedure of Rupp and
Howard-Flanders (19). This method was used in exper-
iments described in Fig. 1 to 3 and Table 2. Method B:
Samples were incubated overnight at 37 °C in 0.4 n
NaOH containing thymine and thymidine, both at 100
µg/ml. After neutralizing with HCl and cooling to 0 °C,
trichloroacetic acid was added to a final concentration
of 5%. After 30 min, the resulting precipitates were
collected on membrane filters and washed with 10 × 5
ml of boiling water. This method was used in experi-
ments described in Tables 3 and 4.

After drying, the discs were placed in toluene scintil-
lational fluid [PPO (2,5-diphenyloxazole), 5 g/liter; 
dimethyl-POPOP (1, 4-bis-2-[4-methyl-5-phenyloxazo-
lyl]-benzene), 0.3 g/liter] for counting in a Packard
526 liquid scintillation spectrophotometer.

RESULTS

Kinetics of DNA transfer. The kinetics of DNA transfer were measured in the mating of
AB3108 Hfr × AB1885 F⁻ to determine a mating period during which Hfr DNA is
transferred to allow its subsequent analysis by density-gradient equilibrium centrifugation. The
donor strain was incubated before mating with
³H-thymine, and DNA transfer was estimated by
determining the ³H remaining in trichloroacetic
acid-precipitable form after elimination of the
donor DNA from the mating mixture by phase
T6 lysis. It is assumed that any increase in re-
main ³H with mating time reflects DNA transfer to the F⁻ cells. In Fig. 1 are shown the amounts of trichloroacetic acid-insoluble ³H
remaining after interruption of mating at the
times indicated. The amounts are expressed as percentages of the initial radioactivity in
the mating mixture.

To measure the efficiency of pair formation, the yields of Pro⁺ Thy⁺ T6² recombinants were
also determined. The pro⁺ gene is transferred early from AB3108. The number of these recom-
binants per 100 Hfr input cells arising after vari-
ous mating periods is included in Fig. 1.

The results show that there is an initial delay in the rate of entry of DNA into female cells. At
mating times longer than 40 min, the amount of T6-resistant, trichloroacetic acid-precipitable
radioactivity increased at a steady rate during the remaining period of measurement.

A mating time of 90 min was selected for sub-
sequent experiments, because DNA transfer is
apparently still occurring at this time and ap-
proximately 2% of the acid-precipitable ³H in the
mating mixture is not liberated by T6 lysis.

Effect of UV irradiation of the Hfr parent on
DNA transfer. A reduction in the number of re-
combinants for a single male marker occurs after
UV irradiation of the excision-defective Hfr
parent AB3108 before mating (27). To examine the extent to which this drop in recombinant
yield reflects a reduced level of DNA transfer, the quantity of ³H-DNA transferred from UV-
irradiated Hfr cells was measured.

A small fraction (0.08 to 0.2%) of the ³H-
DNA of unirradiated Hfr cells was carried
through the phage T6 lysis procedure. This fraction was estimated in each experiment by measuring the residual radioactivity after lysis of a homosexual mating mixture involving an Flac<sup>+</sup> derivative of the T6<sup>+</sup> female strain. Thus, DNA transfer is expressed by subtracting the radioactivity remaining after lysis of the homosexual control mixture from that remaining after lysis of the heterosexual mating mixture.

In preliminary experiments with an Flac<sup>+</sup> derivative of AB1885 in the homosexual control mating, the percentage of <sup>3</sup>H-DNA remaining after phage T6 treatment increased after irradiation of the Hfr cells. This increase, which probably reflects some degradation of the <sup>3</sup>H-DNA of the irradiated Hfr cells (1) and incorporation of breakdown products by the recipients, could be limited to about 0.6% of the initial radioactivity by addition of thymidine to the mating medium. To reduce this incorporation further, the tdk-1 mutation (5, 10) was introduced into the recipient strains. (Cells mutant at tdk lack thymidine kinase and are unable to incorporate thymine or thymidine into DNA.) In subsequent experiments, the trichloroacetic acid-precipitable <sup>3</sup>H remaining after lysis of homosexual control mixtures with UV-irradiated Hfr cells was 0.2 to 0.3% of the initial radioactivity.

The effect of UV irradiating the Hfr uvrB5 derivative on the amount of DNA transferred during mating and on the yield of Pro<sup>+</sup> Thy<sup>+</sup> T6<sup>+</sup> recombinants is shown in Fig. 2. It is apparent that increasing doses of UV irradiation decreases the yield of recombinants more effectively than the amount of DNA transferred.

In the following experiment, in which DNA transferred from a UV-irradiated Hfr was analyzed by density-gradient equilibrium centrifugation, a dose of 200 ergs/mm<sup>2</sup> was chosen. With higher UV doses to the Hfr, the amount of <sup>3</sup>H-DNA transferred was reduced to a level not much greater than the background lysis control, so that analysis of DNA transferred from more heavily irradiated donors was not feasible.

**Analysis of the buoyant density of transferred DNA.** The buoyant density of DNA transferred from UV-irradiated Hfr cells was examined in CsCl density gradients at pH 11.0 to determine whether this DNA is present in recipient cells in a double or single-stranded form. At pH 11.0, the buoyant density of single-stranded DNA is increased, whereas the density of native DNA is unaffected (26). This facilitates differentiation of single- and double-stranded DNA.

The distribution of radioactivity in density gradients containing <sup>3</sup>H-DNA derived either from unirradiated Hfr cells or from F<sup>−</sup> cells, mated with <sup>3</sup>H-thymine-labeled Hfr donors which had been UV-irradiated with 0 or 200 ergs/mm<sup>2</sup>, is shown in Fig. 3. Denatured and native <sup>32</sup>P-marker DNA from AB3108 was added to the gradients in the ratio of 2:1. The recovery of these marker DNA species was in the approximate ratio 1.8:1, showing that there is no substantial loss of single-stranded material. The percentage of the total <sup>3</sup>H-radioactivity in the fractions containing single-stranded marker DNA (Fig. 3A, 15–31) was 10% in the gradient containing DNA from the unmated control and 7% in each of the gradients containing DNA from mating mixtures. Thus, no significant amount of the transferred DNA from either UV-irradiated or unirradiated Hfr cells was detectable in the position corresponding to denatured DNA.

**Mating-dependent DNA synthesis in the recipient.** As shown above, transferred DNA extracted from recipients which had been mated with unirradiated or UV-irradiated Hfr cells appears to be double-stranded. One explanation for this result is that a single strand of DNA is transferred and synthesis of a complementary strand takes place in the recipient during the 90-min mating period. To examine this idea, the technique of Freifelder and Freifelder (3) was adapted for measurement of mating-dependent DNA synthesis in the recipient cells.

Unlabeled cells were mixed in medium containing <sup>3</sup>H-thymine. The Hfr donor carried the tdk-1 mutation to prevent incorporation of thymine. The recipient cells, mutant at thy, drm, and uvrB, were able to incorporate thymine but were UV-irradiated to inhibit replication of the resident DNA during the mating period. UV irradiation of the F<sup>−</sup> cells with 5,000 ergs/mm<sup>2</sup>, a dose approaching that used by Freifelder and Freifelder (3), reduced replication of the F<sup>−</sup> genome to a barely detectable level, but this virtually stopped DNA transfer (Table 2). As a compromise, a dose of 1,000 ergs/mm<sup>2</sup> to the recipient was used, allowing more transfer of DNA but more residual synthesis of F<sup>−</sup> DNA.

Mating-dependent DNA synthesis was measured in the experiments described in Table 3. In these experiments, <sup>3</sup>H-thymine incorporation was measured in a mating mixture of Hfr tdk and UV-irradiated F<sup>−</sup> cells (column II), in a control mixture of Hfr tdk and irradiated F lac<sup>+</sup> cells (III) and in suspensions of irradiated F<sup>−</sup> (IV) and F lac<sup>−</sup> (V) cells incubated separately. The F<sup>−</sup> and F lac<sup>−</sup> strains were isogenic with the exception of the episome. <sup>3</sup>H-uptake by the Hfr cells, incubated at the cell density used in the heterosexual mating mixture, is estimated as the difference between the radioactivity incorporated by the homosexual mating mixture and the F lac<sup>−</sup> cells incubated alone (III-V).
Transfer of DNA

DNA synthesis in the F− is calculated according to the expression (II-IV) – (III-V).

The results in Table 3 show an amount of mating-dependent DNA synthesis in the recipient corresponding to the incorporation of between 850 and 1,600 counts per min per 10^8 Hfr cells, the amount appearing to depend on the initial concentration of the donor cells.

In a similar experiment with 6.7 × 10^7 input Hfr cells per ml, 3H incorporation by the heterosexual mating mixture was measured after lysis of the males by T6. This was compared with the 3H uptake by Hfr and F cells incubated sepa-
rately and then mixed immediately before T6 treatment. The amount of mating-dependent DNA synthesis, calculated from the difference, was 954 counts per min per 10^8 cells. This is consistent with the results reported in Table 3.

When the Hfr cells were UV-irradiated before mating with 200 ergs/mm², mating-dependent DNA synthesis could not be measured.

To compare the amount of DNA transferred with the amount of mating-dependent DNA synthesis in the F⁻ donor, transfer into irradiated female cells was measured. The following modification was made to the growth medium to minimize physiological differences between the Hfr thy dnm and Hfr tdk donors used in the two series of experiments. It has been suggested that the replication velocity of DNA in thymine-requiring strains is lower than that in Thy⁺ strains (17). However, the replication velocity is brought nearer to normal when the thymine-requiring cells are grown in medium containing deoxyguanosine as well as thymine (Zaritsky and Pritchard, manuscript in preparation). Thus, transfer of DNA by Hfr donors was measured after growth of the cells in G medium containing deoxyguanosine at 200 μg/ml and ³H-thymine at 2 μg/ml. These concentrations were maintained in the mating medium so that DNA synthesized in the Hfr during the mating period should also contain ³H-thymine (16). The results of these experiments are shown in Table 4. As the uptake of ³H by the F⁻ and F⁺ tdk cells was similar, DNA transfer has been calculated by subtracting the amount of ³H taken up by the homosexual mating mixture from that taken up by the heterosexual mating.

The amount of DNA transferred from unirradiated Hfr cells to recipients UV-irradiated with 1,000 ergs/mm² corresponded to between 700 and 1,600 counts per min of ³H-thymine per 10^8 Hfr input cells, depending on the concentration of Hfr cells. When these results are compared with those presented in Table 3, it appears that the amount of mating-dependent DNA synthesis in irradiated recipients mated with unirradiated Hfr cells approximately equals the amount of DNA transferred. When the Hfr cells were UV-irradiated with 200 ergs/mm² before mating, the amount of DNA transferred was too small to be measured accurately.

To make these comparisons, it has been assumed that mating pairs are formed with equal efficiency in the crosses BW119 × BW26 and AB3108 × BW40, that the thymine pool in BW26 is not high, and that the thymine-requiring strains BW26 and AB3108 are reduced in ability to synthesize thymine to the same extent.

### TABLE 2. Effect of ultraviolet irradiation of the recipient on DNA transfer from Hfr cells

<table>
<thead>
<tr>
<th>UV dose to recipient (ergs/mm²)</th>
<th>DNA synthesis in F⁻ (% of unirradiated control)</th>
<th>Transfer from Hfr (% ³H in input donor cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>1,000</td>
<td>0.33</td>
<td>0.55</td>
</tr>
<tr>
<td>5,000</td>
<td>0.02</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* BW40 F⁻ tdk uvB5 T66 was UV-irradiated with the doses shown. After sedimentation, the cells were suspended and mated in unlabeled medium for 90 min with AB3108 Hfr thy dnm T66, pregrown with 'H-thymine. DNA transfer was measured for Fig. 2. DNA synthesis in BW26 F⁻ thy dnm uvB5 was measured after UV irradiation by suspending the previously unlabeled cells in G medium containing 'H-thymine (5 μCi/μg). The amount of 'H-thymine incorporated in 90 min was determined.

### TABLE 3. Conjugational DNA synthesis in the F⁻ recipient

<table>
<thead>
<tr>
<th>Input Hfr cells/ml*</th>
<th>³H rise (counts per min per ml)</th>
<th>³H rise due to mating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hfr × F H</td>
<td>Hfr × Flac⁺ HI</td>
<td>F⁻ IV</td>
</tr>
<tr>
<td>12.0</td>
<td>2,341</td>
<td>1,409</td>
</tr>
<tr>
<td>8.6</td>
<td>1,700</td>
<td>1,195</td>
</tr>
<tr>
<td>8.3</td>
<td>1,833</td>
<td>1,072</td>
</tr>
<tr>
<td>7.0</td>
<td>1,873</td>
<td>1,269</td>
</tr>
<tr>
<td>6.0</td>
<td>1,609</td>
<td>781</td>
</tr>
</tbody>
</table>

* Figures shown to be multiplied times 10².

Calculated from (H⁻ IV)-H⁻ V-1. Results are expressed as counts per minute per 10^8 Hfr cells. BW119 Hfr tdk uvB5 was washed by sedimentation and then mixed with either BW26 F⁻ thy dnm uvB5 or an Flac derivative, BW126, both UV-irradiated with 1,000 ergs/mm² as for Table 2. The mixture contained (per ml) about 9.5 × 10^7 F⁻ or F⁺ cells and 'H-thymine (5 μCi), 2 μg. For the solo incubations, G medium containing thymine was substituted for the Hfr culture. Each line refers to a separate experiment. ³H-uptake was measured after 90 min of incubation. Under these conditions of counting, 1 μCi of ³H gave 4.3 × 10³ counts per min.

### DISCUSSION

DNA transferred during conjugation from unirradiated Hfr donors was found to have the...
buoyant density of double-stranded DNA (Fig. 3). Assuming that only a single strand of DNA is transferred (16, 20), it follows that its conversion to double-stranded material occurs in the female. Conversion may involve synthesis of a complement to the transferred strand or may result from the complexing of the incoming DNA with the F- genome in some form of recombinant structure.

To distinguish between these two possibilities, we have measured DNA synthesis occurring as a consequence of mating in UV-irradiated, excision-defective females. The amount of DNA synthesis occurring when these females were mated with unirradiated Hfr donors corresponded approximately to the amount of DNA transferred. This synthesis presumably reflects formation in the recipient of a complement to the transferred strand of Hfr DNA rather than a nonspecific stimulation of DNA synthesis in the F- during mating. Freifelder and Freifelder (3), using a similar experimental system, detected 10 times the control amount of DNA synthesis in F- cells after transfer of F- DNA into recipients UV-irradiated with over 8,000 ergs/mm². However, a dose of 5,000 ergs/mm² to the F- bacteria in our experiments reduced transfer from Hfr donors to a barely detectable level (Table 2). Possible explanations for this difference are that irradiation of the F- parent reduces transfer from Hfr donors more effectively than from males harboring an autonomous sex factor, that transferred F- DNA is replicated more than once in the irradiated recipient cells, or that the F- strain used in our experiments behaves differently from that used by Freifelder and Freifelder.

Analysis of the buoyant density of DNA transferred from UV-irradiated Hfr cells suggests that this is also double-stranded in the recipient. The exogenote cannot be a conserved duplex with dimers in both strands, because the transferred lesions are not susceptible to repair by the excision process (8, 23, 27). Thus, the exogenote contains a single strand of DNA transferred from the Hfr. It is unlikely that this is converted to double-stranded material solely by recombination with the F- DNA for the following reason. UV irradiation of excision-defective Hfr cells reduces the frequency with which male markers are recovered in recombinants (23, 27), so that some segments of the transferred material will not be integrated into the recipient genome. We have found no evidence for single-stranded fragments of Hfr DNA in the recipient. It is improbable that the unincorporated DNA was completely degraded by the end of the 90-min mating period and so escaped detection, because the amount of transferred DNA measurable at this

TABLE 4. Measurement of DNA transferred by unirradiated Hfr donors

| Input Hfr cells/ml | Hfr × F | Hfr × F lac- | Hfr-trans- 
|-------------------|---------|-------------|-----------------
<table>
<thead>
<tr>
<th>× 10⁴</th>
<th>H- resistant T6 lysis</th>
<th>H- thymine</th>
<th>ferred (counts per min per 10⁷ Hfr cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Mo</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>8.0</td>
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<td>6.3</td>
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</table>

* Figures shown to be multiplied times 10⁴.

* Calculated from (III-III) × 10⁴. Results expressed as counts per minute per 10⁴ Hfr cells. AB3108 Hfr thy dnm uvrB5 T6² was grown in medium containing deoxyguanosine (200 µg/ml) and H- thymine (2 µg/ml) at the indicated specific activity. The cells were washed by sedimentation. They were then mated with BW40 T tdk uvrB5 T6² or an F lac- derivative, BW140, both UV-irradiated with 1,000 ergs/mm² and at about 10⁴ cells/ml. Deoxyguanosine and H- thymine were added to the mating mixture at the concentrations mentioned above. The amounts of H- resistant T6 lysis were measured after 90 min of incubation. Each line refers to a separate experiment. T6-resistant H- incorporated by the irradiated F- and F' cells was measured in the second and third experiments and amounted to 178 and 202 counts per min, respectively.

Time was less effectively reduced by UV irradiation of the Hfr cells than was the yield of recombinants (Fig. 2).

A more probable explanation, suggested elsewhere (8, 27), is that transferred dimer-containing DNA is replicated during mating like DNA transferred from unirradiated Hfr donors. It was not possible to determine whether mating-dependent DNA synthesis occurs in the recipient after irradiation of the Hfr cells. To do this, it was necessary to inhibit replication of the F- DNA by UV irradiation, and irradiation of both parents reduced DNA transfer to a level which could not be determined accurately.

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LITERATURE CITED


