EFFECTS OF 5-BROMODESOXYURIDINE UPON GENE RECOMBINATION IN *ESCHERICHIA COLI* K-12¹

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GENETIC analyses of crosses of *E. coli* K-12 Hfr $\times$ F[—] have demonstrated that factors possessed by the donor parent (Hfr) are transferred in a characteristic, oriented, and progressive manner to the recipient (F[—]) parent (WOLLMAN, JACOB, and HAYES 1956), and that recombinants segregate only from the F[—] parent (LEDERBERG 1956). The transfer of radiophosphorous-containing material (probably DNA) has been correlated with the transfer of genetic factors by GAREN and SKAAR (1958), while experiments of JACOB and WOLLMAN (1954) and PARDEE, JACOB and WOLLMAN (1958) indicate that little, if any, cytoplasmic transfer occurs during conjugation. It is for these reasons that *E. coli* K-12 has proven to be a particularly suitable system in which to test the effects of agents, such as ultraviolet irradiation or radiophosphorous disintegration, upon genetic recombination (JACOB and WOLLMAN 1958). If the donor of genetic material is treated with these agents and permitted to conjugate with appropriate F[—] cells, nonspecific physiological effects can be resolved from those which affect the genetic material.

By subjecting donor bacteria to low doses of ultraviolet irradiation, significant increases in the frequency of gene recombination have been obtained by JACOB and WOLLMAN (1958). These authors assert that the mechanism best explaining their data is one based upon the partial replica hypothesis (LEVINTHAL 1954). According to this hypothesis, a new recombinant segment of genetic material arises by alternately copying from one, then the other, of two paired homologous strands. They suggest that lesions are formed upon ultraviolet treated genetic material, which represent sites along its length and interfere with normal replication by causing the newly formed strand to switch back to the nonirradiated segment for completion of replication.

Since the mode of action of ultraviolet irradiation is incompletely understood, these experiments do not test directly the partial replica hypothesis. Other agents whose action upon genetic material can be more clearly inferred are desirable to test this hypothesis.

The nucleic acid base analogue 5-bromouracil is specifically incorporated into

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the DNA of *E. coli* B and quantitatively replaces thymine (DUNN and SMITH 1954; ZAMENHOF and GRIBOFF 1954). This analogue, administered as the free base or its desoxyriboside, can act as an inducer of specific mutations for phage T4B (BENZER and FREESE 1958) and is mutagenic for phage T2 (LITMAN and PARDEE 1956) and for thymine requiring strains of *E. coli* (ZAMENHOF, DE-GIOVANNI and GREER 1958). In light of these observations, studies of the effects of 5-bromouracil DNA upon genetic recombination were initiated. It is the purpose of this paper to describe the results of genetic experiments upon *E. coli* K-12 Hfr $\times$ F⁻ crosses in which the donor bacteria were grown under conditions of thymine starvation favoring incorporation of the analogue.

MATERIALS AND METHODS

Strains of *E. coli* K-12 HfrC and P678 were obtained from F. JACOB. HfrC bears the markers *thr*⁺ *leu*⁺ *azi-s* *T1-s* *lac*⁺ *gal*⁺ λ *str-s* and is a derivative of HfrH of HAYES (1957). P678 bears the markers *thr* *leu* *azi-r* *T1-r* *lac*- *gal*- λ -*s* *str-r* *xyl*- *mtol*- (ability to synthesize threonine *thr*, leucine *leu*, resistance (*r*) or sensitivity (*s*) to sodium azide *azi*, streptomycin *str*, bacteriophage *T1* or λ ; fermentation of lactose *lac*, galactose *gal*, xylose *xyl*, or mannitol *mtol*). Strain P678 was treated with phage T6 and a T6 resistant mutant recovered for use in kinetic experiments. The order of transfer of markers from donor HfrC to recipient P678 is *thr*⁺ *leu*⁺ *azi-s* *T1-s* *lac*⁺ *gal*⁺ with *thr*⁺ *leu*⁺ at the origin (WOLLMAN *et al.* 1956). Streptomycin sensitivity and markers concerned with fermentation of xylose and mannitol are transferred with low frequency.

Nutrient agar slants: containing per liter, 8 gm DIFCO dehydrated nutrient broth, 5 gm NaCl, and 20 gm agar. *M-26 basal minimal medium*: (after W. R. SISTROM) contained per liter, K₂HPO₄ 8.7 gm, KH₂PO₄ 6.8 gm, NH₄Cl 1.0 gm, Na₂SO₄ 0.1 gm, MgCl₂·6H₂O 0.16 gm, CaCl₂·2H₂O 0.002 gm, FeCl₃·6H₂O 0.002 gm, glucose 4.0 gm, and thiamin 0.001 gm. *Sulfanilamide medium*: (after BENZER and FREESE 1958) consisted of M-26 plus, per liter, Ca-pantothenate 1 mg, pyridoxine 1 mg, thiamin 1 mg, xanthine 25 mg, hypoxanthine 25 mg, vitamin-free casein hydrolysate 1.0 gm, sulfanilamide 2.0 gm, and uracil 1 mg. *Selective and scoring media*: M-26 1.5 percent agar plates containing 160 mg per liter streptomycin (MS) were used to select *thr*⁺ *leu*⁺ *str-r* recombinants. M-26 1.5 percent agar plates with 4 gm per liter galactose replacing the glucose, 80 mg per liter L-threonine, 40 mg per liter L-leucine, and 160 mg per liter streptomycin (MGTLS) were used to select *gal*⁺ *str-r* recombinants; the same medium without threonine and leucine (MGS) was used to select *thr*⁺ *leu*⁺ *gal*⁺ *str-r* recombinants. Nutrient agar plates containing 100 mg per liter sodium azide or about 10⁹ particles of phage T1 were employed to score for azide and T1 sensitivity or resistance, respectively. Fermentation abilities were scored using EMB complete medium supplemented either with lactose or galactose (LEDERBERG 1947).

Vitamin-free casein hydrolysate was obtained from Nutritional Biochemicals Co., streptomycin from C. Pfizer and Co., and 5-bromodesoxyuridine from the California Foundation for Biochemical Research.

Crosses of HfrC $\times$ P678 were standardized according to the following protocol. Dilute inocula from nutrient agar slants were spread upon plates of M-26 supplemented with threonine and leucine, incubated at 37°C overnight, and stored at 10°C. From these plates a single colony of each parent was suspended in 20 ml liquid M-26 (plus threonine and leucine for P678) and incubated overnight. Twenty ml amounts of sulfanilamide medium were inoculated with 0.2 ml of the overnight Hfr culture and incubated ten to 11 hours at 37°C without aeration. One portion of sulfanilamide medium contained 50 μ gm per ml thymine (control) while others contained various concentrations of 5-bromodesoxyuridine (BD). Simultaneously with the inoculation of the sulfanilamide medium, 20 ml portions of M-26 supplemented with threonine and leucine were inoculated in a similar manner with P678 F⁻ and incubated as above. A sample of each parent was then spread upon each type of selective medium (about 10^7 cells per plate). Both parents were centrifuged separately, washed three times with glucose-free M-26, and resuspended to a concentration of about $2-5 \times 10^8$ cells per ml. Total cell counts were made with a Petroff-Hausser counting chamber under dark field illumination at 800 $\times$. To determine colony-forming ability, plate counts of the HfrC parent were prepared by spreading appropriate dilutions onto M-26 agar plates. The bacteria were mixed in the proportions of $1-2 \times 10^7$ HfrC per ml to $2-5 \times 10^8$ P678 per ml. A small amount of nutrient broth concentrate and glucose were added to a final concentration of 0.1 and 0.01 percent, respectively. Five ml of the mating mixture was pipetted into a prewarmed 125 ml flask and incubated on a slow speed shaking water bath (70, $\frac{3}{4}$ inch strokes per minute) at 37°C. Sixty minutes after mixing, samples of the mating mixture were withdrawn, diluted, and plated on the selective media (MS for *thr*⁺ *leu*⁺ *str*-r, MGTLs for *gal*⁺ *str*-r, and MGS for *thr*⁺ *leu*⁺ *gal*⁺ *str*-r colonies). Recombinant colonies were counted 36 hours after plating. To remove background contamination and to aid in scoring, a sample of 100 to 200 colonies from MGS and MGTLs was spotted onto identical media. The replicas were incubated 12 hours, then replica plated onto suitable scoring media.

The number of recombinant colonies per 100 initial HfrC cells, as determined by total cell counts, will be termed the *recombination frequency*, while the number of recombinants of a given selected genotype bearing a designated HfrC marker among 100 recombinants of this type will be termed the *genetic constitution* of the recombinants (after WOLLMAN *et al.* 1956).

Total cell counts, rather than plate counts, were employed in all determinations of recombination frequency, since the viability of BD-treated cells was variable and low. For some crosses, the frequency of *thr*⁺ *leu*⁺ *str*-r colonies recovered exceeded the plate counts of the treated HfrC parent, indicating that cells which no longer can form colonies on M-26 agar plates can donate their genetic material.

To determine the extent of replacement of thymine by 5-bromouracil, DNA from analogue-treated donor cells (60 μ gm BD per ml) was isolated by the method of HOTCHKISS (1957), hydrolyzed one hour in 12 *N* perchloric acid at 100°C (MARSHAK and VOGEL 1951), and the resulting bases were chromato-

grammed in the solvent of MACNUTT (1952) which permitted simultaneous resolution of adenine, guanine, cytosine, thymine and 5-bromouracil. The bases were located under ultraviolet illumination, cut out, eluted in 0.1 *N* HCl and their amounts determined by the maximal extinction technique of DUNN and SMITH (1957). The average replacement of thymine by 5-bromouracil for two samples of DNA, each analyzed in triplicate, was 49 percent. Thus, incorporation of BD into HfrC DNA is extensive under the conditions of growth reported here.

RESULTS

Genetic constitution of gal⁺ str-r colonies: When colonies bearing proximal markers, such as *thr⁺ leu⁺ (str-r)*, are selected from the mating mixture, genetic material consisting of segments of various sizes will be transferred to the recipient bacteria. Some of these segments will bear all of the more distal markers such as *lac⁺* and *gal⁺*, while others will not. This effect may be normalized by employing selection for distal markers and examining the inheritance of markers proximal to this. When selection for *gal⁺ str-r* colonies is employed, the entire segment from *gal⁺* to the origin must have been transferred, and only effects upon integration will be manifest. The genetic constitution of *gal⁺ str-r* recombinants (Table 1) shows a decrease in the frequency of markers integrated from BD-treated donors. This result can best be accounted for by assuming that portions of the HfrC genetic material which are integrated into the zygote are decreased.

This assumption is verified by a consideration of the percent of *gal⁺ str-r* colonies which bear all four of the HfrC unselected markers (Table 2). Here, a striking decrease of this type of colony is evident among matings involving BD-treated donors.

Recovery of recombinant colonies: When the HfrC parent was grown in thymine (control) or various concentrations of BD, comparison of the number of *thr⁺ leu⁺ str-r*, *gal⁺ str-r*, or *thr⁺ leu⁺ gal⁺ str-r* colonies per 100 initial HfrC

TABLE 1

Genetic constitution of gal⁺ str-r colonies; from each mating 180 to 250 colonies were examined

Exp. no.	Treatment of HfrC	Percent HfrC markers integrated			
		<i>thr⁺ leu⁺</i>	<i>azi-s</i>	<i>T1-s</i>	<i>lac⁺</i>
I	Control	83	88	63	69
	12μ gm/ml BD	71	85	38	58
	60μ gm/ml BD	58	69	44	46
	120μ gm/ml BD	63	70	37	51
	Control	69	58	53	64
II	12μ gm/ml BD	71	55	51	58
	24μ gm/ml BD	55	47	48	49
	60μ gm/ml BD	58	44	47	56
	Control	80	59	57	70
III	60μ gm/ml BD	66	35	40	49

TABLE 2

Frequency of gal⁺ str-r colonies bearing all four HfrC unselected markers

Exp. no.	Treatment of HfrC	Percent <i>gal⁺ str-r</i> colonies bearing all four unselected HfrC markers
I	Control	49.5
	12 μ gm/ml BD	25.0
	60 μ gm/ml BD	19.9
	120 μ gm/ml BD	27.7
II	Control	44.4
	12 μ gm/ml BD	33.2
	24 μ gm/ml BD	29.6
	60 μ gm/ml BD	30.5
III	Control	40.0
	60 μ gm/ml BD	16.0

cells yields the relationship between the dose of BD and the recombination frequency shown in Table 4. The marked decrease in the frequency of all three classes of recombinant colonies can be related to the concentration of BD. The decrease of *thr⁺ leu⁺ str-r* colonies is similar to that of *gal⁺ str-r* colonies and suggests that transfer is not the stage affected. The *thr⁺ leu⁺* markers of the HfrC donor are closely linked and penetrate the recipient cell at about eight minutes from the time of mixing the two parents and are, therefore, proximal to the HfrC marker *gal⁺*, which penetrates the F⁻ cell at about 25 minutes (WOLLMAN *et al.* 1956). A greater decrease of distal to proximal markers would be expected if transfer were the affected stage. The decrease of proximal markers is nearly equal to that of distal markers, clearly indicating that these results are not due to interference with transfer of genetic material but to some effect upon its integration into the zygote or upon the ability of HfrC to form effective contacts. The greater decrease of *thr⁺ leu⁺ gal⁺ str-r* recombinants relative to either *thr⁺ leu⁺ str-r* or *gal⁺ str-r* indicates that one effect of BD is upon integration since the simultaneous integration of two widely separated markers is reduced more than that of either factor alone.

A second, lethal, effect of BD is also manifest when the frequencies of recombinant colonies issuing from matings in which the donor was BD-treated are compared to the control values. This is, that the decrease is of a greater magnitude than is the effect upon the integration of markers. For instance, in Experiment II (Table 4) the *thr⁺ leu⁺ str-r* colony frequency is decreased by a factor of about six, still a consideration of the percent of nonselected markers integrated (Experiment II, Tables 1 and 3) shows a decrease by a factor of about 0.20. Thus, many of the analogue treated cells are dead in the sense that they no longer can transfer their genetic material or form effective contacts.

Genetic constitution of thr⁺ leu⁺ str-r colonies: Table 3 presents the genetic constitution with respect to the nonselected markers of *thr⁺ leu⁺ str-r* colonies formed from BD-treated and control donor bacteria. A decrease in the frequency of factors integrated from BD-treated donor bacteria is apparent in each in-

TABLE 3

Genetic constitution of thr⁺ leu⁺ str-r colonies; 180 to 250 colonies from each mating were examined. The HfrC of Exp. IV was a lac⁻ derivative of HfrC obtained by ultraviolet irradiation

Exp. no.	Treatment of HfrC	Percent HfrC markers integrated			
		azi-s	T1-s	lac ⁺	gal ⁺
II	Control	90	73	43	27
	12 μ gm/ml BD	80	65	44	25
	24 μ gm/ml BD	78	62	26	14
	60 μ gm/ml BD	78	60	32	18
III	Control	86	65	41	26
	60 μ gm/ml BD	76	51	19	16
IV	Control	91	70	..	20
	60 μ gm/ml BD	74	54	..	6

TABLE 4

The effect of various concentrations of BD upon the recovery of recombinant colonies. For each value 400 to 1,500 colonies were counted

Exp. no.	Dose of BD μ gm/ml	thr ⁺ leu ⁺ str-r per 100 Hfr	gal ⁺ str-r per 100 Hfr	thr ⁺ leu ⁺ gal ⁺ str-r per 100 Hfr	Percent viable cells	
					a*	b†
I	Control	13.20	3.74	...	3.53	98
	12	8.50	2.66	...	3.20	100
	60	3.38	0.88	...	3.84	57
	120	2.24	0.56	...	4.00	49
II	Control	3.89	1.32	...	2.95	93
	12	1.35	0.46	...	2.94	56
	24	1.13	0.27	...	4.18	52
	60	0.75	0.18	...	4.16	34
V	Control	15.00	3.62	2.95	4.16	5.10
	12	1.60	0.46	0.24	3.56	6.67
	60	0.73	0.13	0.08	5.66	9.13
	120	0.17	0.03	0.02	5.66	8.50

* a—The ratio of thr⁺ leu⁺ str-r to gal⁺ str-r colonies.

† b—The ratio of thr⁺ leu⁺ str-r to thr⁺ leu⁺ gal⁺ str-r colonies.

stance. This decrease indicates that the processes of integration or transfer may be affected, however, as the thr⁺ leu⁺ str-r/gal⁺ str-r ratio remained unchanged (see Table 4) it is more probable that integration is the affected stage. If, say, the rate of breakage during transfer were to have been increased, this ratio should also increase.

Kinetic experiments: The HfrC donor was grown in sulfanilamide medium containing either thymine or BD, mated to P678 T6-r, and aliquots of the mating mixture were periodically removed, treated with T6 (multiplicity of 100) and KCN (2×10^{-3} M) in order to stop transfer of genetic material and to destroy the donor parent (HAYES 1957; FISHER 1957). Estimation of the genetic consti-

tution of *thr*⁺ *leu*⁺ *str-r* colonies permitted a determination of the times of entry of the various HfrC markers into recipient cells.

The times of entry and plateau levels of the unselected markers are presented in Table 5 and Figures 1 and 2. It is clear that one effect of the analogue is to lengthen slightly the time required for transfer. Since the HfrC parent requires energy for transfer of genetic material (FISHER 1957), the increase in the time required for transfer may be a nonspecific effect due to the analogue upon the donor cells. The lower values for the percent of nonselected markers integrated obtained from the mating of BD-treated cells can be explained by interference with the processes of integration. Were transfer to be affected by the analogue's increasing the rate of breakage, the plateau levels would be decreased; in addi-

TABLE 5

Results of kinetic experiments: For each cross and sample 100 colonies were scored

Treatment of HfrC	<i>azi</i> -s	<i>T1</i> -s	<i>lac</i> ⁺	<i>gal</i> ⁺	
50 μ gm per ml thymine	88	66	30	11	plateau levels (in percent)
60 μ gm per ml BD	10	10	25	35	times of entry (in minutes)
60 μ gm per ml BD	79	39	10	6	plateau levels (in percent)
60 μ gm per ml BD	20	25	35	45	times of entry (in minutes)

Samples of the mating mixture were taken at ten minute intervals and treated as described. The plateau levels represent the genetic constitution of the *thr*⁺ *leu*⁺ *str-r* colonies and are averages of values obtained from several samples of the mating mixture taken after the markers had reached their maximum frequency of integration. The times of entry are accurate to the nearest five minutes.

FIGURE 1.—The kinetics of transfer of different markers. The genetic constitution of *thr*⁺ *leu*⁺ *str-r* colonies formed at the times indicated has been analyzed, and these values plotted as a function of time. Only the times of appearance of the *azi*-s and *T1*-s markers have been graphed; *lac*⁺ and *gal*⁺ have been omitted for clarity (see Figure 2). Solid lines represent control values; dotted lines, values obtained from matings in which the donor was grown with 60 μ gm per ml 5-bromodesoxyuridine (see Table 5).

FIGURE 2.—The kinetics of transfer of different markers. The genetic constitution of *thr⁺ leu⁺ str-r* colonies formed at the times indicated has been analyzed, and these values plotted as a function of time. Here, only the times of appearance of the *lac⁺* and *gal⁺* markers have been graphed; see Figure 1 for *azi-s* and *T1-s*. Solid lines represent control values; dotted lines, values obtained from matings in which the donor was grown with 60 μ gm per ml 5-bromodesoxyuridine (see Table 5).

tion, the rate at which a plateau is attained would be decreased, which is not the case. Were integration the affected stage, only the plateau levels would be decreased, which is the observed result. Further proof that integration is affected is evident when transfer effects are ruled out by selection for distal markers and analysis of the unselected markers transferred before the distal markers (see Tables 1 and 2).

Control experiments: The possibility remained that the genetic effects described were due to treatment of the donor with sulfanilamide and directly resulted from conditions of thymine starvation and not from replacement in the DNA of thymine by bromouracil. To test this, HfrC was grown in sulfanilamide medium containing neither BD nor thymine, mated to P678, and the genetic constitution of resultant *thr⁺ leu⁺ str-r* recombinants was examined. No differences were observed between these results and those of concomitant control matings. For effects upon integration to be manifest, BD must at least be present in the sulfanilamide medium. Prolonged conditions of thymine starvation did lead to a marked decline in the frequency of recombination. This decline is due to death of the treated donors and their inability to form effective contacts, or to transfer their genetic material.

The possibility that BD-treated HfrC bacteria underwent mutation at the loci employed for these genetic experiments is remote. To explain the observations on this basis many simultaneous mutations at a number of specific loci must be invoked. Nevertheless, this possibility was tested. BD-treated HfrC bacteria were mated and genetic information obtained (see Experiment III in Tables 1, 2, and 3). A portion of the unmated HfrC bacteria was retained and plated onto all four scoring media at a concentration of about 5×10^7 cells per plate. These plates were incubated and examined the following day. No mutations were observed from the HfrC genotype but for several *T1-r* colonies which were also present when thymine-grown cells were plated.

DISCUSSION

The above results clearly demonstrate that a decrease in the frequency of HfrC markers recovered among recipient cells is due to an effect of the analogue upon some stage of integration of the genetic material. This is evident when a distal marker, *gal*⁺, is selected and the recovery of markers proximal to it, which must have been transferred, is examined (Table 1). Furthermore, when genotypes of colonies bearing the distal marker are compared, those colonies which were derived from matings of BD-treated donors bearing all four HfrC markers were reduced by a factor of two (Table 2). To explain the decrease in frequency of this kind of *gal*⁺ colony, the average number of switches between *thr*⁺ *leu*⁺ and *gal*⁺ has to be increased by 0.7 (suggestion of PROFESSOR C. LEVINTHAL). If the frequency of *thr*⁺ *leu*⁺ *azi-s* *T1-s* *lac*⁺ *gal*⁺ *str-r* colonies among all *gal*⁺ *str-r* colonies selected is symbolized by *a*, and if one assumes no switches must occur between *thr*⁺ *leu*⁺ and *gal*⁺ to yield this kind of colony, then the average number of switches occurring in that region is *n*, where:

$$e^{-n} = a$$

For control crosses e^{-n} is 0.49 and *n* is 0.69, while in BD crosses e^{-n} is 0.25 and *n* is 1.39. Thus, the average number of switches is increased from 0.69 to 1.39, or by a factor of two.

Other effects of the analogue, too, are present and include death of BD-treated cells in the sense that their ability to transfer genetic material or to form effective contacts is lost. These effects are seen most clearly in the results presented in Table 4 where the ability of BD-treated donors to form recombinants decreases at a greater rate than the loss of colonies bearing simultaneously all HfrC markers (Table 2).

The analogue also affects transfer, increasing the time required for markers to enter the recipient. This effect was resolved from that upon integration as a transfer effect would be negated when distal markers were selected. If a mating between an Hfr donor and F⁻ recipient bacterium is considered from the viewpoint that the transferred genetic material is rescued and integrated into the F⁻ genome by recombination, the presence in treated donors of BD appears to result in the integration of smaller regions of the transferred genetic material. This effect can best be interpreted by the partial replica hypothesis of LEVINTHAL (1954) and

JACOB and WOLLMAN (1955, 1958) in which integration of genetic material proceeds by pairing of homologous segments and the formation of a new replica between them. This replica may copy alternate regions of the paired segments by switching from one to the other. When one of the two paired parental segments is altered, in this case by the presence of Hfr bromouracil DNA, copying is forced away more frequently to the other parental (F^-) segment. The presence of the analogue in Hfr DNA appears to act in much the same fashion, but not to the extreme degree as ultraviolet treated genetic material (JACOB and WOLLMAN 1955, 1958; WOLLMAN *et al.* 1956).

Two hypotheses can be presented to account for the observed increase in the frequency of recombinant events. In the first, pairing could be disrupted so that partial replicas can only be formed from the paired regions of Hfr genetic material. The second assumes pairing to remain unaffected and requires that sites along the length of the Hfr genetic material discourage the formation of partial replicas. An experiment to distinguish between these two hypotheses is in progress. If the former hypothesis is true, it is predicted that the frequency of recombinant types from closely linked markers will decrease; if the latter, an increase in the frequency of recombinant types is expected.

Recently, EPHRAI-ELIZUR and ZAMENHOF (1959) observed that transforming ability of bromouracil DNA of *B. subtilis* is present, but the activity is either decreased or unaltered depending on the markers observed. These results are not incompatible with those presented in this paper. Both may be explained by the hypothesis that rescue of markers present on bromouracil DNA occurs by the mechanism of partial replica formation in which the replicas are biased away from the genetic material containing the analogue.

BENDICH, PAHL and BROWN (1957), in studies on the chromatographic fractionation of *E. coli* DNA containing C^{14} -5-bromouracil, have demonstrated that the DNA is heterogenous with respect to the incorporation of 5-bromouracil. Their work raises an important consideration in the interpretation of the results of this paper. Does transferred genetic material actually contain the analogue, or does the bromouracil-containing DNA remain in the donor, and is the effect a more subtle one? From the observed great extent of replacement of thymine by bromouracil it appears unlikely that one third of the genome of HfrC donors can remain free of the analogue; and the transferred genetic material represents one third of the content of a haploid nucleus (STENT and FUERST 1959). The heterogeneity of 5-bromouracil incorporation is most likely due to a populational heterogeneity of the analogue-treated cultures.

SUMMARY

The effects of the thymine analogue 5-bromodesoxyuridine upon genetic recombination in *Escherichia coli* K-12 have been investigated. When Hfr donor bacteria are treated with the analogue under conditions of thymine starvation and mated to untreated recipient bacteria, both the stages of transfer and integration of genetic material are affected by the analogue. The effects of the analogue

upon each stage have been resolved. The average number of switches occurring over a given region is increased by a factor of two. Analysis of the genetic constitution of recombinant progeny suggests that the rescue of factors contained on the transferred genetic material is hindered so that smaller portions are integrated. These results can be best explained by the partial replica hypothesis of LEVINTHAL and JACOB and WOLLMAN.

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