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Incorporation and Excision of 5-Fluorouracil from Deoxyribonucleic Acid in *Escherichia coli*†

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When *Escherichia coli* are grown in the presence of 5-fluorouracil, the 5-fluorouracil is incorporated almost exclusively into ribonucleic acid as fluorouridylate. In this study, small but detectable amounts were incorporated into ribonucleic acid as fluorocytidylate and into deoxyribonucleic acid as fluorodeoxyuridylate and fluorodeoxycytidylate. The amount of 5-fluorouracil found in deoxyribonucleic acid as fluorodeoxyuridylate increased 50-fold when the cells were deficient in both deoxyuridine triphosphatase and uracil-deoxyribonucleic acid glycosylase activities. Therefore, the same mechanisms which excluded uracil from deoxyribonucleic acid in vivo also excluded 5-fluorouracil. Even though purified uracil-deoxyribonucleic acid glycosylase excised 5-fluorouracil from deoxyribonucleic acid at only 5% the rate with which it excised uracil, most of the 5-fluorouracil excised from deoxyribonucleic acid in vivo was apparently excised directly by uracil-deoxyribonucleic acid glycosylase rather than by repair initiated by excision of uracil.

Heidelberger (6) has reviewed evidence which indicates that 5-fluorouracil is incorporated into nucleic acids in bacteria and mammals only in place of uracil in RNA. It has been assumed that 5-fluorouracil is taken up and converted to 5-fluorouridine 5'-triphosphate (5'-fluoro-UTP) in cells by the same pathway by which uracil is converted to UTP, and that the RNA polymerase does not discriminate well between UTP and 5-fluoro-UTP (Fig. 1). The existence of this common pathway has permitted the isolation of mutants resistant to 5-fluorouracil which contain altered enzymes at the various steps in the pathway (17). Although Kaiser and Kwong have more recently reported finding very small amounts of fluorocytosine in RNA (8), the very low level indicates either that RNA polymerase can distinguish between CTP and 5-fluoro-CTP, or that 5-fluoro-UTP is not a good substrate for CTP synthetase. No incorporation of 5-fluorouracil or 5-fluorocytosine into cellular DNA has been previously reported.

The failure to incorporate 5-fluorouracil into DNA indicates that 5-fluoro-UDP is not a substrate for ribonucleotide reductase, that DNA polymerase can discriminate between 5-fluoro-dUTP and dTTP, or that mechanisms operating to exclude uracil from DNA also exclude 5-fluorouracil. Since *Escherichia coli* DNA polymerases I and III discriminate poorly between dUTP and dTTP (18, 21), and ribonucleotide reductase has low base specificity (10), the latter explanation seems most likely. Furthermore, Lozeron and Szybalski (13) have demonstrated that 5-fluorouracil is incorporated into phage DNA in PBS1-infected *Bacillus subtilis*, suggesting that the biochemical alterations permitting the incorporation of uracil into DNA in these infected cells also permit the incorporation of 5-fluorouracil into DNA, but at the time of their experiments the nature of these alterations was not fully known.

It has now been shown that *E. coli* mutants deficient in both dUTPase (*dut* mutation) and uracil-DNA glycosylase (*ung* mutation) do incorporate uracil into their DNA (22). The DNA of mutants deficient in only one of these enzymes contains little or no uracil (H. Warner, unpublished data). In addition, phage PBS2 induces the synthesis of proteins to inactivate host dUTPase and uracil-DNA glycosylase activities (5, 9, 19), suggesting that both of these activities must be absent to permit the synthesis of uracil-containing phage DNA. We have used the *E. coli dut, ung,* and *dut ung* mutants to reinvestigate the incorporation of 5-fluorouracil into DNA to determine whether the exclusion of 5-fluorouracil from DNA is mediated by the same mechanisms which exclude uracil.

MATERIALS AND METHODS

Materials. 5-[6-^{14}C]fluorouracil was obtained from New England Nuclear Corp. and had a specific activity of 12.9 mCi/mmol. [6-^{3}H]uridine was obtained from Schwarz/Mann and was diluted with unlabeled uridine to give a final specific activity of 4,000 mCi/mmol. 5-[6-^{3}H]fluorouracil was obtained from Schwarz/Mann

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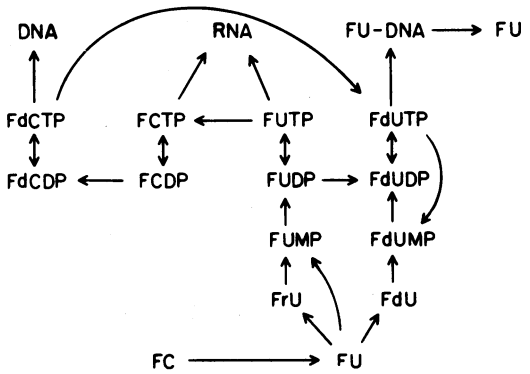

FIG. 1. *Putative pathway for uptake and incorporation of 5-fluorouracil into nucleic acids in E. coli.*

and was diluted with unlabeled 5-fluorouracil to give a final specific activity of 4,200 mCi/mmol. All commercial enzymes were obtained from Worthington Biochemical Corp.

Bacteria. The bacteria used in these experiments were two isogenic sets of strains (Table 1). The BD1153, 1154, 1156, 1157 series was constructed by Bruce Duncan, and the BW274, 275, 276, 277, 278 series was obtained from Bernard Weiss. All incorporation experiments were carried out in the medium of Davis and Mingioli (3), supplemented with 2 μg each of nicotinic acid and thiamine per ml, and 5 mg of Casamino Acids per ml. For the experiment with the BW270 series the cells were first grown at 25°C in medium containing 1.0% tryptone, 0.5% yeast extract, and 1.0% NaCl, since BW278 grew poorly in Davis medium at any temperature. The cells were then harvested, washed, and suspended in Davis medium for the incorporation experiment.

Incorporation of 5-[6-^{14}C]fluorouracil into nucleic acids. Deoxyadenosine was added to *E. coli* cultures (about 3 × 10^8 cells/ml) to give a final concentration of 100 μg of deoxyadenosine per ml. Then 5-[6-^{14}C]fluorouracil (specific activity = 12.9 mCi/mmol) was added to give a final concentration of 0.125 μCi/ml, and the cultures were shaken for 1 h at 37°C (BD strains) or for 2 h at 25°C (BW strains). The nucleic acids were precipitated by adding the cultures to 0.1 volume of 50% trichloroacetic acid, and the amount of fluorouracil incorporated into RNA and DNA was determined (20). The DNA was hydrolyzed in formic acid, the free bases were separated by paper chromatography in 86% butanol, and the paper was cut up and counted to locate the radioactivity (20). Standards were cochromatographed with the samples in every lane.

Base analysis of RNA and DNA labeled with 5-[6-^{3}H]fluorouracil. *E. coli* BD1154 and BD1157 were grown at 37°C in Davis medium to a concentration of 1 × 10^8 to 2 × 10^8 cells/ml. To 50 ml of cells, deoxyadenosine and 5-[6-^{3}H]fluorouracil (specific activity, 4,200 μCi/μmol) were added to give final concentrations of 500 μg/ml and 2 μCi/ml, respectively. The cultures were shaken for 60 min at 37°C, and the cells were harvested by centrifugation. The labeled cells were suspended in 15 ml of 0.9% KCl containing about 6 × 10^{10} unlabeled cells, and the cells were again harvested by centrifugation. The cells were suspended in 10 ml of 1× SSC (0.15 M sodium chloride plus 0.015 M sodium citrate) containing 50 mg of sodium dodecyl sulfate. These suspensions were stirred for 2 h at room temperature to lyse the cells, 5 mg of pronase was added, and incubation was continued for 15 min at 37°C. This solution was treated with an equal volume of phenol to denature protein, and two volumes of ethanol were layered onto the aqueous layer. The ethanol was mixed in with a stirring rod to spool the DNA (15), and the remaining precipitate (RNA) was collected by centrifugation. The RNA was dissolved in 5 ml of 1× SSC, precipitated with 2 volumes of ethanol, and finally dissolved in 5 ml of 1× SSC.

TABLE 1. *E. coli strains used*

Strain	Genotype	Source/reference
BD1137	*thi-1 argH1 nadB4 purI66 pyrE41 lacY1 malA1 xyl-7 rha-6 ara-13 gal-7 rpsL9 tonA2* or *A22 supE44* T2^r *rel-1?*	4
BD1154	BD1137 *nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction
BD1153	BD1137 *ung-1 nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction
BD1156	BD1137 *dut-1 nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction
BD1157	BD1137 *ung-1 dut-1 nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction
BW274	KL16 *dut-1*	B. Weiss
BW275	KL16 *ung-1 Δxth*	B. Weiss
BW276	KL16 *dut-1 ung-1 Δxth*	B. Weiss
BW277	KL16 *Δxth*	B. Weiss
BW278	KL16 *Δxth dut-1*	B. Weiss

A 1-ml amount of the RNA solution was incubated for 30 min at 30°C with 0.05 mg of RNase (heated 10 min at 80°C to inactivate deoxyribonuclease activity [15]) and then chromatographed on a Sephadex G-50 column (1.5 by 25 cm) equilibrated with 0.01 M triethylamine bicarbonate solution. The fractions in the major radioactive peak, corresponding to short oligonucleotides, were combined, lyophilized, and redissolved in 1 ml of 1× SSC. A 0.1-ml (BD1154) or 0.2-ml (BD1157) amount of these solutions was diluted to 0.9 ml with water, 0.1 ml of 1 M 2(*N*-morpholino)-ethanesulfonic acid buffer (pH 6.0) and 0.1 U of spleen phosphodiesterase were added, and the mixture was incubated for 2 h at 37°C to convert the oligonucleotides to 3′-ribonucleotides. This mixture was then chromatographed on a DEAE-Sephadex A25 column (1.1 by 20 cm) equilibrated with 0.05 M ammonium formate (pH 3.7), and the ribonucleotides were eluted with a linear gradient generated from 160 ml each of 0.05 and 0.20 M ammonium formate (pH 3.7). The total recoveries of radioactivity applied were 91 and 92% for BD1154 and BD1157, respectively.

The spooled DNA was dissolved in 5 ml of 1× SSC and incubated with 0.2 mg of RNase (heated) for 30 min at 30°C to digest any contaminating RNA. The DNA was respooled twice and finally dissolved in 2.5 ml of 0.1× SSC. A 1-ml amount of this DNA solution was incubated for 15 min at 37°C with 50 μmol of Tris chloride buffer (pH 8.0), 20 μmol of $MgCl_2$, 0.1 mg of pancreatic deoxyribonuclease, 0.5 mg of bovine serum

albumin, and 0.2 U of venom phosphodiesterase, and then heated for 2 min at 100°C. The 5'-deoxyribonucleotides were separated on a DEAE-Sephadex A25 column as described above for the 3'-ribonucleotides. The total recoveries of radioactivity applied were 78 and 89% for BD1154 and BD1157, respectively.

Preparation of substrate for uracil-DNA glycosylase. *E. coli* BD1157 (6 ml) was infected with a mutant of phage T5 unable to induce dUTPase activity (23), and the culture was shaken vigorously for 15 min at 37°C. Deoxyadenosine was added to the culture (final concentration, 100 μg/ml) followed by 5-[6-^{3}H] fluorouracil (specific activity = 4,200 mCi/mmol; final concentration = 4 Ci/ml). The culture was shaken for another 5 h to complete lysis, and the phage were then purified by centrifugation in cesium chloride step gradients (23). The DNA was extracted from the phage by treatment with phenol. Phage DNA containing radioactive uracil was prepared in a similar manner after labeling with [6-^{3}H]uridine.

Uracil-DNA glycosylase assay. Uracil-DNA glycosylase was purified about 4,800-fold from *E. coli* by procedures similar to those described by Lindahl et al. (12). The final preparation had a specific activity of 1.86×10^5 U of protein per mg and a concentration of 5×10^5 U/ml. For the experiments described here this enzyme was diluted 1,000-fold in 0.03 M potassium phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol, and 5% glycerol. One enzyme unit is the amount of enzyme required to release 1 nmol of uracil from phage PBS2 DNA in 30 min at 37°C.

The assay mixtures containing 26.7 μmol of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.8), 0.35 μmol of EDTA, 1 μmol of dithiothreitol, 6 μmol of sodium chloride, 0.6 μmol of sodium citrate, 10 μg of bovine serum albumin, heat-denatured DNA (about 1,500 cpm in fluorouracil or 1,150 cpm in uracil), and purified uracil-DNA glycosylase in a total volume of 0.25 ml were incubated at 37°C. The amount of fluorouracil or uracil released was determined by a modification of the column assay described earlier (4). The reaction was stopped by adding 0.75 ml of 0.1 M ammonium formate buffer (pH 4.3). At pH 4.3 neither uracil nor 5-fluorouracil binds to the anion-exchange column, whereas the DNA substrate does. When the usual assay was used to assay the release of 5-fluorouracil from DNA, no product was detected because the 5-fluorouracil, which has a pK at about 8 (25), remains bound to the column.

RESULTS

An isogenic set of *E. coli*, differing only in the *dut* and *ung* genes, was grown for 60 min in the presence of 5-[6-^{14}C]fluorouracil, and the DNA was separated from RNA by incubation in 0.3 N NaOH for 16 h at 30°C (20). The amount of radioactivity remaining in the DNA and in the acid-soluble ribonucleotides was determined (Table 2). Although deoxyadenosine was always present to enhance the incorporation of 5-fluorouracil into DNA by the salvage pathway, about 99.9% of the radioactivity is always incorporated into RNA. Some radioactivity can be detected in the DNA from *ung-1*, *dut-1*, and wild-type *E. coli* strains, but the amount in the DNA from *E. coli dut-1 ung-1* is much greater, particularly when based on the incorporation into RNA. The *dut ung* and *dut* mutants grow more slowly than the other strains, so there is less overall incorporation of the fluorouracil into nucleic acids in these strains.

The DNA was then hydrolyzed, and the free bases were separated by paper chromatography and analyzed for radioactivity (Fig. 2). Only the *dut ung* DNA contains a large amount of 5-fluorouracil (note the 10-fold larger scale for BD1157). All of the other samples contained a small amount of radioactivity in the region corresponding to 5-fluorouracil, and an equivalent amount in the region corresponding to 5-fluorocytosine. The data clearly indicate that only when the cells are deficient in both dUTPase and uracil-DNA glycosylase is there considerable incorporation of 5-fluorouracil into DNA. They also indicate that 5-fluorocytosine is incorporated into DNA and that this incorporation is independent of the activities of these two enzymes.

The presence of 5-fluorocytosine in the DNA suggests that some 5-fluoro-UTP must be converted to 5-fluoro-CTP by CTP synthetase, and that 5-fluoro-CDP is then reduced to 5-fluoro-dCDP which is converted to the triphosphate and incorporated into DNA. However, preliminary attempts to detect 5-[6-^{14}C]fluorocytosine in RNA were unsuccessful. To increase the sensitivity of the assay, the incorporation experiment was repeated with 5-[6-^{3}H]fluorouracil. Since the hydrogen at the 6 position of fluorouracil is extensively exchanged during both alkaline hydrolysis and formic acid hydrolysis, the RNA and DNA were separated and degraded to nucleotides enzymatically. The nucleotides were then separated on DEAE-Sephadex (Fig. 3) and the results of these chromatograms are summarized in Table 3. Whereas 5-fluorocytosine can be detected in the RNA, the ratio of 5-fluorouracil to 5-fluorocytosine was extremely high

TABLE 2. *Incorporation of 5-[^{14}C]fluorouracil into nucleic acids in E. coli*

Strain	Genotype	Total radioactivity[a]		% cpm in DNA
		DNA (×10^2)	RNA (×10^5)	
BD1154		4.04	1.38	0.29
BD1153	*ung-1*	2.20	1.41	0.16
BD1156	*dut-1*	0.77	0.98	0.08
BD1157	*dut-1 ung-1*	17.8	0.99	1.80
BW274	*dut-1*	0.87	1.66	0.05
BW278	*dut-1 Δxth*	1.87	1.03	0.18
BW276	*dut-1 ung-1 Δxth*	17.1	1.31	1.30

[a] Expressed as total counts per minute in the nucleic acid per milliliter of culture.

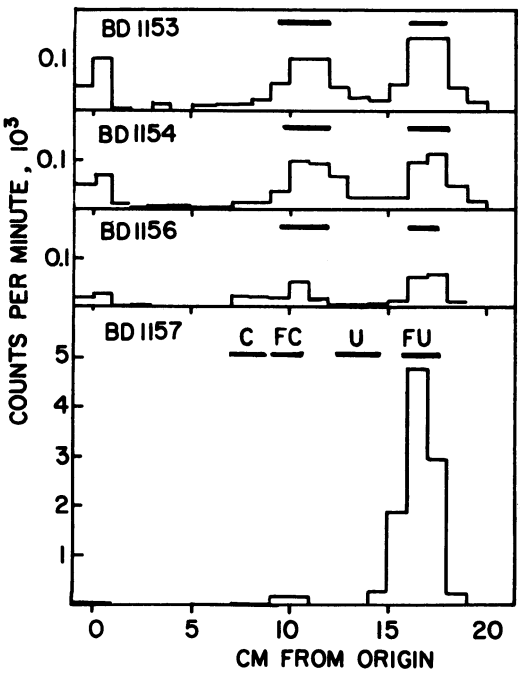

FIG. 2. *Base composition of 5-fluorouracil-labeled E. coli DNA. The DNA was hydrolyzed to free bases which were separated by paper chromatography. The chromatograms were cut into 1-cm pieces which were counted to locate the radioactivity. The horizontal bars indicate the location of pyrimidine standards, particularly 5-fluorocytosine (FC) and 5-fluorouracil (FU), in each lane.*

(more than 150) in the RNA from both the *dut*$^+$ *ung*$^+$ cells and the *dut ung* cells. In contrast, in the DNA from *dut*$^+$ *ung*$^+$ cells this ratio was only 1.4, because of the efficient removal of 5-fluorouracil from this DNA in vivo. This ratio increased to 12 when the DNA from *dut ung* cells was analyzed. The 5-fluorocytosine incorporated into DNA appears to be quite stable and not influenced by the genotype of the cells used in these experiments.

Since any DNA containing 5-fluorouracil will also contain uracil, it is possible that the 5-fluorouracil is not removed from the DNA by direct action of the uracil-DNA glycosylase, but is removed indirectly during base-excision repair of the uracil-containing regions (11). To test this possibility, 5-fluorouracil- and uracil-containing T5 DNAs were prepared and incubated with purified uracil-DNA glycosylase, and the ratio of release of free uracil and 5-fluorouracil were measured. Both uracil and 5-fluorouracil were excised from the DNA as free bases by uracil-DNA glycosylase, but the release of uracil is 20-fold faster than the rate of release of 5-fluorouracil (Fig. 4).

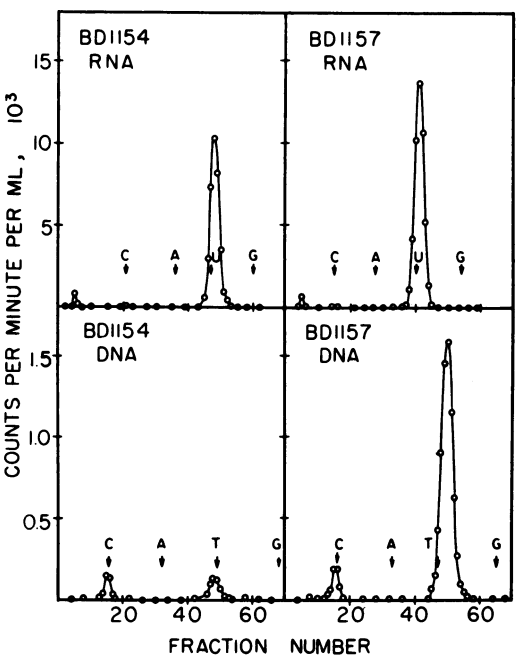

FIG. 3. *Chromatography of nucleotides on DEAE-Sephadex A-25. The 3'-ribonucleotides and 5'-deoxyribonucleotides produced by enzymatic degradation of 5-fluorouracil-labeled RNA and DNA were eluted with a 0.05 to 0.2 M gradient of ammonium formate (pH 3.7). The arrows indicate the fractions in the chromatograms corresponding to each of the usual nucleotides found in the nucleic acids, determined by absorbance at 260 and 280 nm. The samples chromatographed correspond to 20 ml of culture for deoxyribonucleotides, and 1 ml (BD1154) or 2 ml (BD1157) of culture for ribonucleotides.*

TABLE 3. *Distribution of radioactivity in nucleic acids in E. coli labeled with 5-[^{3}H]fluorouracil*[a]

E. coli	DNA			RNA		
	Radioactivity[b]		FU/FC	Radioactivity[b]		FU/FC
	FdUMP	FdCMP		FUMP	FCMP	
BD1154 (*dut*$^+$ *ung*$^+$)	102	73	1.4	136,200	810	168
BD1157 (*dut ung*)	1,355	112	12.1	92,740	515	180

[a] F, Fluoro.
[b] Expressed as total counts per minute in the nucleic acid per milliliter culture.

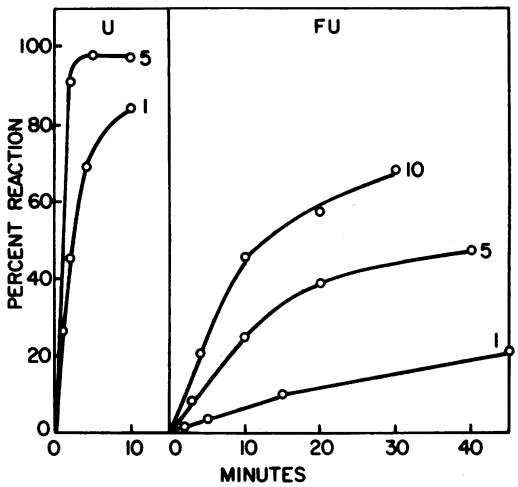

FIG. 4. *Activity of purified uracil-DNA glycosylase on uracil- (U) and 5-fluorouracil-(FU)containing DNA. Various amounts of diluted enzyme were added to incubation mixtures, and samples were removed at various times and assayed for the amount of radioactivity released as uracil or 5-fluorouracil. The numbers on the curves indicate the microliters of diluted enzyme used. The results are plotted as the percentage of the total uracil or 5-fluorouracil present in the DNA which was excised during the incubation.*

The presence of an atom larger than hydrogen on position 5 of the uracil ring probably accounts for the slower release of 5-fluorouracil from DNA compared with the rate of release of uracil. However, the electron-withdrawing effect of the fluoro group lowers the pK of the phenol group on position 4 (25), thereby causing this group to partially dissociate at the pH of the assay. When the assay was done at various pH's, the release of 5-fluorouracil from DNA was not relatively better at low pH (Fig. 5), suggesting that the spatial interference of the fluorine atom at position 5 is more important than the ionization of the phenolic group at position 4.

The above results indicate that 5-fluorouracil, which is incorporated into DNA in dUTPase-deficient *E. coli*, can be at least partially removed by the glycosylase enzyme. However, the slower rate suggests that excision of 5-fluorodeoxyuridylate during base excision repair of uracil-containing DNA in vivo may also contribute to the absence of 5-fluorouracil in the DNA of dUTPase-deficient strains. To test this possibility, an experiment similar to that described in Table 2 and Fig. 2 was repeated, using strains carrying a deletion in the *xth* gene. This gene codes for exonuclease III, which has an associated endonuclease activity which is specific for apurinic and apyrimidinic sites (AP sites) in DNA (26). This enzyme accounts for about 90% of the AP endonuclease activity in *E. coli* (14). After incision at AP sites by AP endonuclease, nucleotide excision and repair synthesis occurs, presumably by the action of DNA polymerase I (11). Taylor and Weiss have shown that *dut xth* mutants are not viable at high temperature, whereas *dut ung xth* mutants are viable (24). Presumably, the *dut xth* mutants accumulate AP sites in their DNA which go unrepaired, leading to cell death, whereas in the *dut ung xth* mutant these sites are formed slowly, if at all. Since nucleotide excision should be slower in *xth* mutants, excision of fluorouracil as 5'-fluoro-dUMP after removal of uracil by uracil-DNA glycosylase should also be reduced. The amount of 5-fluorouracil found in BW278 (*dut xth*) was only slightly higher than that found in BW274 (*dut*), whereas a much larger amount is found in BW276 (*dut ung xth*) (Table 2 and Fig. 6). Therefore, the uracil-DNA glycosylase itself is apparently directly responsible for the removal of almost all of the 5-fluorouracil from the DNA of these cells. Results obtained with BW275 (*ung*

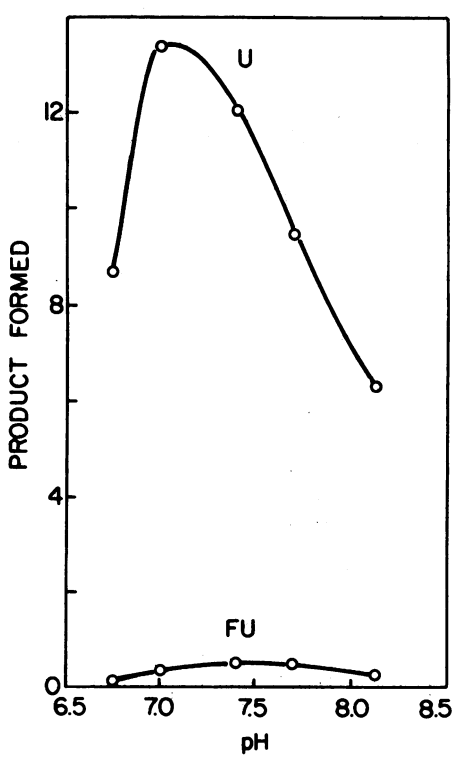

FIG. 5. *Effect of pH on excision of uracil and 5-fluorouracil from DNA by purified uracil-DNA glycosylase. The results are plotted as the counts per minute ($\times 10^2$) in uracil (U) or 5-fluorouracil (FU) excised per microliter diluted enzyme per 5 min of incubation.*

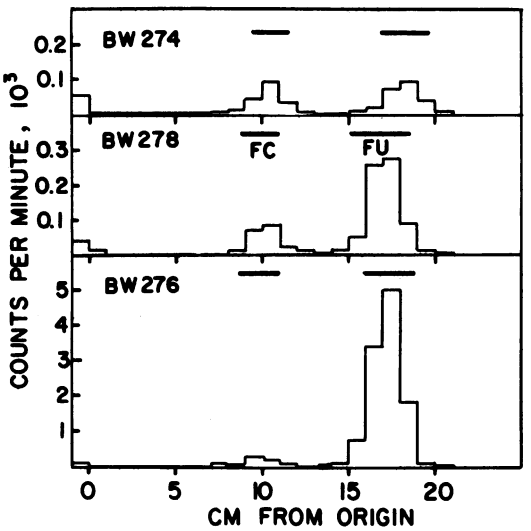

FIG. 6. *Base composition of 5-fluorouracil-labeled E. coli DNA. The results were obtained as indicated for Fig. 2. The phenotypes of the strains used are BW274 (dut), BW278 (dut xth), and BW276 (dut xth ung).*

xth) and BW277 (*xth*) were similar to those obtained with BW274 (*dut*) and are not shown.

DISCUSSION

The results presented here indicate that the same enzymes that exclude uracil from DNA are responsible for the exclusion of 5-fluorouracil as well. The relative rate of activity of dUTPase on 5-fluoro-dUTP and dUTP is not known, but the uracil-DNA glycosylase is about 5% as active on fluorouracil-containing DNA as on uracil-containing DNA. This reduced activity is sufficient to excise most of the 5-fluorouracil as rapidly as it is incorporated. The presence of any substituent other than hydrogen on the 5 position of the uracil is apparently sufficient to reduce the activity of the glycosylase, and the fluoro group appears to be the only substitution which allows any detectable activity. 5-Bromouracil and thymine are not excised by the enzyme (12).

The conditions used here for growing the *E. coli dut ung* mutant normally result in a replacement of about 13% of the thymine by uracil (H. Warner, unpublished data). This replacement may be even higher in the presence of fluorouracil which will be converted to 5-fluoro-dUMP, an inhibitor of thymidylate synthetase. Although the exact amount of 5-fluorouracil per micromole of deoxyribonucleotide in DNA was not determined in these experiments, the value can be estimated from the specific activity of the 5-fluorouracil used and the amount of DNA per milliliter of culture. From the data given in Table 2, and assuming complete recovery of the DNA after alkaline hydrolysis and reprecipitation of the DNA with trichloroacetic acid, it can be estimated that only about 1 to 2% of the thymine has been replaced by 5-fluorouracil in these cells. The effect of this fluorouracil on the properties of the DNA are not known from these experiments. Lozeron and Syzbalski (13) have already shown that PBS2 phage containing 5-fluorouracil in place of 10 to 20% of the uracil are slightly more sensitive to UV light than are normal phage containing only uracil, so 5-fluorouracil in DNA in place of thymine would presumably also increase the sensitivity of the DNA to UV light. The presence of 5-fluorouracil in *E. coli* DNA could lead to occasional miscoding as observed in the case of RNA containing 5-fluorouracil (2). Herrington and Takahashi (7) have observed that incorporation of 5-fluorouracil into PBS2 DNA is mutagenic, presumably due to such miscoding.

The much lower ratio of fluorouracil to fluorocytosine in the DNA of *dut ung* mutants compared with the ratio in the RNA suggests that some excision of 5-fluorouracil from the DNA is occurring. Although it is possible that a repair pathway other than base excision repair is operating to specifically remove 5-fluorouracil but not 5-fluorocytosine from the DNA, it seems more likely that residual uracil-DNA glycosylase activity is present. T. Lindahl has found that as much as 0.5% of the normal glycosylase activity may remain in the *E. coli ung-1* mutant (personal communication).

It is clear from the results that both RNA and DNA contain small amounts of 5-fluorocytosine when 5-fluorouracil is added to the growth medium. Kaiser and Kwong (8) previously found that RNA isolated from *E. coli* cells grown in the presence of 0.1 to 0.2 μmol of 5-fluorouracil per ml contains as much as 1.0 mol% 5-fluorocytosine; the content of 5-fluorocytosine in RNA in our experiments was much less when the cells were grown in the presence of only 0.5 nmol of 5-fluorouracil per ml. The presence of 5-fluorocytosine in RNA indicates that 5-fluoro-UTP is converted to 5-fluoro-CTP in *E. coli*, but at a rate probably less than 1% that of the conversion of UTP to CTP as estimated by the ratio of fluorouracil to fluorocytosine in the RNA. In contrast to these results, Bean and Tomasz reported finding fluorocytosine-containing nucleotides in *Diplococcus pneumoniae*, but did not detect any fluorocytosine in the RNA (1).

Some of the fluoro-CTP synthesized in vivo must also be converted to fluoro-dCTP, presumably at the diphosphate level by ribonucleotide

reductase. The route of conversion of 5-fluorouracil to fluoro-dUTP in this experiment is less clear, since at least three possibilities exist: (i) reduction of fluoro-UDP to fluoro-dUDP, (ii) uptake of 5-fluorouracil by the thymine salvage pathway (17), and (iii) deamination of fluoro-dCTP by deoxycytidine triphosphate deaminase (16). This latter pathway can make only a minor contribution to the fluoro-dUTP pool because of the small amount of fluoro-CTP apparently formed, but the first two pathways probably both make major contributions. Our experiments were always done in the presence of deoxyadenosine to enhance uptake and incorporation of 5-fluorouracil into DNA by the salvage pathway, and we have not determined how much is incorporated into DNA in the absence of deoxyadenosine. What is clear from the data in Fig. 2, 3, and 6 is that only in *dut ung* cells is 5-fluorouracil incorporated into DNA and does it remain there in any significant quantity.

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