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Some Properties of Transfer Ribonucleic Acids from 5-Fluorouracil-Treated *Escherichia coli**

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ABSTRACT: The composition and some of the functions of transfer ribonucleic acid from 5-fluorouracil-treated *Escherichia coli* have been examined. The analog can replace as much as 70% of the uracil in transfer ribonucleic acid. This substitution has little effect on the proportions of the other major nucleotide components: the purine to pyrimidine ratio remains unchanged. Transfer ribonucleic acid from 5-fluorouracil-inhibited cells is, however, deficient in several minor base components. The pseudouridylic acid content decreases from 2.7 to 0.99 mole %; this decrease is proportional to the extent of analog incorporation. The level of ribothymidylate in this ribonucleic acid is also reduced. Despite these changes in nucleotide composition the capacity of 5-fluorouracil-containing transfer ribonucleic acid to accept amino acids remains essentially the same as that of normal transfer ribonucleic acid. The mixture of transfer ribonucleic acid isolated from cells exposed to the analog can be partially separated into normal and 5-fluorouracil-containing components by chromatography on columns of methylated albumin silicic acid, with resolution increasing as the pH of the eluting buffer is raised from 5.2 to 8.0.

The 5S ribosomal ribonucleic acid of 5-fluorouracil-treated cells also contains the analog; 35% of the uracil is replaced by 5-fluorouracil. This ribonucleic acid remains associated with the ribosome and does not contaminate the transfer ribonucleic acid fraction.

The structural and functional changes brought about by the introduction of a base analog into RNA can provide important insights into the active centers of the molecule. It has been known for some time that FU[1] will replace uracil in bacterial RNA (Horowitz and Chargaff, 1959) as well as in the RNA of higher organisms and viruses (reviewed by Heidelberger, 1965). Previous investigations have examined the inhibition of ribosome formation by the analog (see review by Osawa, 1965; Andoh and Chargaff, 1965; Hills and Horowitz, 1966) and the effects of FU incorporation into mRNA on protein synthesis (Heidelberger, 1965; Horowitz and Kohlmeier, 1967). Few studies, however, have concerned themselves with the effects of FU on tRNA. The results reported here deal with this aspect of FU action and describe the effects of the fluoropyrimidine on some of the properties and functions of the tRNA from *Escherichia coli*. It was found that FU can replace up to 70% of the uracil in tRNA and that this RNA was deficient in at least two minor pyrimidine constituents, pseudouridylic and ribothymidylic acids. Despite these changes FU-tRNA was readily able to accept amino acids, in most cases at least as efficiently as normal tRNA. FU was also incorporated into the low molecular weight (5S) rRNA; this remained associated with ribosomes. A preliminary account of these findings has appeared (Horowitz and Huntington, 1967) and Lowrie and Bergquist (1968) have since described similar observations.

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[1] Abbreviations used are: FU, 5-fluorouracil; FU-tRNA, tRNA isolated from 5-fluorouracil-treated *E. coli;* MAK, methylated bovine serum albumin adsorbed on kieselguhr; MASA, methylated bovine serum albumin adsorbed on silicic acid.

Materials and Methods

Bacterial Growth. E. coli strain B-RA, kindly supplied by Dr. Rakoma Wiesner, was grown in a glucose–salts medium (Demerec and Cahn, 1953) either in flasks with aeration by shaking or, when larger quantities were needed, in 15-l. fermentors (New Brunswick Scientific Co.). 5-Fluorouracil (15–50 μg/ml) and thymidine (10–25 μg/ml) were added when the cells reached exponential growth. After incubation for the indicated time periods, the cultures were chilled and the cells were harvested by centrifugation, washed twice with 0.01 M Tris-HCl buffer (pH 7.4) containing 0.01 M magnesium acetate (Standard buffer), frozen, and stored at −20° until used. Sodium azide (0.01 M) was added to stop further synthetic activity, in those experiments which examined the effect of duration of FU treatment on the nucleotide composition of tRNA.

Preparation of RNA. Cells were broken by grinding with alumina (Alcoa A-305) and the ribosomes carefully separated from the supernatant by ultracentrifugation (see Hills and Horowitz, 1966, for details). tRNA was prepared from the ribosome-free supernatant by a modification (Hills and Horowitz, 1966) of the phenol–sodium dodecyl sulfate procedure of Kurland (1960). This RNA was further purified by chromatography on DEAE-cellulose or MASA or by an isopropyl alcohol fractionation step as described by Zubay (1962). Amino acids were stripped from tRNA by incubation at 37° in 1.8 M Tris-HCl buffer (pH 8.0) for 90 min (Sarin and Zamecnik, 1964).

5S rRNA was prepared from the ribosomal pellet by the phenol–sodium dodecyl sulfate method. The majority of the high molecular weight rRNA was removed by precipitation with 1–2 M NaCl and the 5S RNA further purified by chromatography on Sephadex G-100 using 1 M NaCl to elute the RNA (Schleich and Goldstein, 1966).

Chromatographic Procedures. Chromatography on MASA columns was carried out as described by Okamoto and Kawade (1963). The columns were equilibrated with either 0.02 M sodium acetate buffer (pH 5.2) or 0.05 M Tris-HCl buffer (pH 8.0). Gel filtration on 0.9 × 150 cm columns of Sephadex G-100 was performed as described by Schleich and Goldstein (1966) using 1 M NaCl to elute the RNA. tRNA was purified on DEAE-cellulose as described by Holley *et al.* (1961); the RNA was eluted with a linear gradient of NaCl in standard buffer.

In each instance, RNA was detected by measuring the absorbance at 260 mμ. Radioactive samples were precipitated with 7% trichloroacetic acid after the addition of 0.6–1.0 mg of bovine serum albumin as carrier. The precipitates were washed, dissolved in 1 N ammonium hydroxide, and evaporated onto planchets which were then counted in a Nuclear-Chicago D47 gas-flow counter equipped with micromil window.

Analytical Methods. Nucleotide analyses were carried out on RNA samples hydrolyzed with 0.5 N NaOH at 37°. After 2 days the hydrolysate was neutralized with HCl, and any insoluble material was removed by centrifugation. The nucleotides were separated by paper chromatography (Whatman No. 1) using the one-dimensional procedure developed by Lane (1963), which separates the four major nucleotides and fluorouridylic acid or the two-dimensional method described by Lipshitz and Chargaff (1960), which separates pseudouridylic acid but not fluorouridylic acid from uridylic acid. In the two-dimensional procedure, the amounts of uridylate and fluorouridylate were estimated by the following equations (at pH 7.0): uridylate (μmoles/ml) = $[6.55(A_{260}) - 7.8(A_{280})]/38.2$ and fluorouridylate (μmole/ml) = $[10(A_{280}) - 3.5(A_{260})]/38.2$. RNA was determined by means of the orcinol reaction (Mejbaum, 1939) and phosphorus by the method of King (1932).

Amino Acid Acceptor Activity. Aminoacylation of tRNA was carried out essentially as described by Kelmers *et al.* (1965) or Pestka (1966), except that the reaction mixtures were supplemented with 0.001 M CTP. Aminoacyl-tRNA synthetase fractions free of tRNA were prepared by the method of Kelmers *et al.* (1965) in early experiments and as described by Muench and Berg (1966) in later ones. After incubation at 37° for 20 min, the reaction mixtures were chilled, and the reaction was stopped by the addition of 7% trichloroacetic acid; 0.5–1.0 mg of yeast RNA was added as carrier. Radioactivity in the precipitate was determined as described earlier. The values reported were corrected for the amount of label incorporated in controls containing no tRNA. Under our conditions, incorporation was linearly dependent upon tRNA up to a concentration of 1.2 mg/ml. The label precipitated by cold trichloroacetic acid could be solubilized by heating in 7% trichloroacetic acid at 90° for 10 min and by the stripping procedure used to remove amino acids from tRNA (Sarin and Zamecnik, 1964).

Materials. 5-Fluorouracil was generously provided by Dr. W. E. Scott of Hoffman-LaRoche, Inc. Carbon-labeled compounds were purchased from either the California Corp. for Biochemical Research or New England Nuclear Corp. ATP and CTP were obtained from P-L Biochemicals, Inc.; bovine albumin (fraction V) from Pentex, Inc., and DEAE-cellulose (Cellex-D) from Bio-Rad Laboratories.

Results

Nucleotide Composition of FU-tRNA. tRNA from FU-inhibited *E. coli* has a high percentage of its uridylic acid component replaced by fluorouridylate. This is demonstrated in Table I which presents the nucleotide composition of tRNA isolated from cells exposed to the analog for various time periods up to 3 hr. There was a progressive increase in the degree of substitution of FU for uracil; after 3 hr, more than 70% of the uridylic acid had been replaced. This substitution had little effect on the proportion of the other major nucleotide components, and the purine to pyrimidine ratio remained constant (Table I). Similar results were obtained with either of the two procedures employed for nucleotide analysis, method I which separates fluorouridylic acid from uridylic acid and method II which separates pseudouridylic acid, but not fluorouridylic acid, from uridylate (see Methods). However, higher

TABLE I: Nucleotide Composition of tRNA from FU-Treated *E. coli*.[a]

Duration of FU Treatment	Method of Nucleotide Anal.	Nucleotide Constituent (mole %)							FU ×100 / U + FU	pu/Py
		A	C	G	U	FU	ψ	U + FU		
0	I	19.6	30.2	32.2	18.0			18.0		1.07
	II	19.3	30.0	31.8	16.2		2.7	16.2		1.05
45	I	19.4	28.8	31.1	11.5	9.3		20.8	45	1.02
	II	19.4	29.9	31.8	9.3	8.2	1.6	17.4	47	1.05
90	I	19.5	29.3	31.2	9.0	11.1		20.1	55	1.03
	II	19.9	30.2	31.0	7.4	10.1	1.4	17.5	58	1.04
180	I	19.4	30.0	31.0	6.8	12.8		19.6	65	1.02
	II	19.8	29.3	31.7	4.8	13.5	0.99	18.3	74	1.06

[a] Nucleotides were separated by paper chromatography as described by Lane (1963), method I, or Lipshitz and Chargaff (1960), method II. See Methods for details.

values for uridylic acid were always obtained with the one-dimensional chromatographic procedure (method I), undoubtedly because uridylate and pseudouridylic acid were not separated by this procedure.

While the relative amounts of the major base constituents remained unchanged, except for the substitution of FU for uracil, the pseudouridylic acid content of tRNA progressively decreased as the time of exposure to FU increased and, after 3 hr, was reduced from 2.7 mole % to less than 1 mole % (Table I). Comparison of the results from a number of experiments indicated that the decrease in pseudouridylic acid content was approximately proportional to the degree of replacement of uridylate by fluorouridylate (Table II).

The effect of FU on another minor pyrimidine constituent of tRNA, ribothymidylic acid, was also examined, using the two-dimensional paper chromatographic technique of Hayashi *et al.* (1966) to separate this component. A marked reduction was noted in the amount of ribothymidylate in FU-tRNA compared with that in normal tRNA. In fact so little of this nucleotide could be detected in tRNA from cells exposed to FU for 2 hr that it was impossible with our methods to accurately quantitate the amount present.

Chromatographic Examination and Fractionation of FU-tRNA. FU-tRNA consists of a mixture of FU-containing tRNA and normal, unsubstituted tRNA present in the cells before FU was added to the culture. Several attempts were made to separate these two species of RNA by gel filtration and other chromatographic procedures. Our tRNA preparations were also examined closely to determine whether the low minor pyrimidine base content of FU-tRNA could be ascribed to the presence of 5S rRNA or high molecular weight rRNA breakdown products, which contain none or only relatively small amounts of these minor base components (Rosset *et al.*, 1964; Osawa, 1960).

No contamination of the tRNA preparations with other RNA species was observed by chromatography on columns of MASA and only minor amounts of acid-soluble material were detected (Figure 1). It was, however, noted that the FU-tRNA peak was broader than the normal and skewed toward the trailing edge (compare Figure 1A, B), an indication that some separation between FU-containing and normal tRNA occurred. This was confirmed by the experiments in Figure 2 which show the elution pattern obtained when FU-tRNA labeled with [^{14}C]FU was chromatographed together with a tenfold excess of unlabeled normal tRNA; the normal tRNA, represented by the ultraviolet absorption profile, was eluted before the analog-containing tRNA. At pH 5.2, normal tRNA came off the MASA columns at *ca.* 0.65 M NaCl, while FU-tRNA was eluted at *ca.* 0.75 M NaCl (Figure 2A). The degree of separation increased as the pH was raised, with best results obtained at pH 8.0 where the two RNA species were almost completely resolved (Figure 2B). Partial separations with MAK column chromatography have previously been reported (Sueoka and Yamane, 1963; Andoh and Chargaff, 1965).

Both normal and FU-tRNA were eluted from DEAE-cellulose at 0.5 M NaCl; no separation of the two RNA species was observed with the procedure used in these studies (see Methods). Chromatography on DEAE-

TABLE II: Relationship between FU Incorporation and the Pseudouridylic Acid Content of tRNA.

Expt	FU × 100 / FU + U	% Decrease in ψ	Expt	FU × 100 / FU + U	% Decrease in ψ
1	38	33	5	58	50
2	47	43	6	67	67
3	48	33	7	74	64
4	52	53			

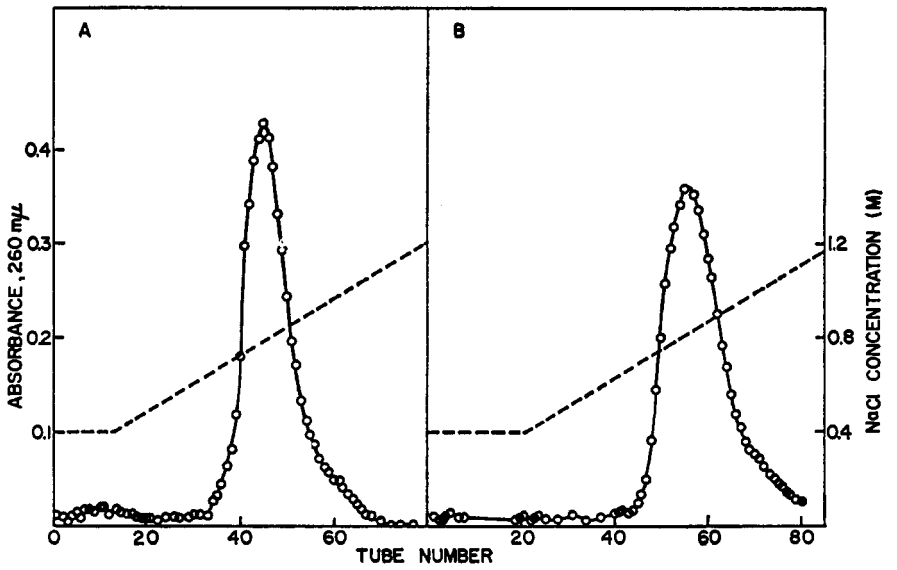

FIGURE 1: Chromatography of tRNA on columns of MASA. The RNA was eluted with a linear gradient of NaCl in 0.02 M sodium acetate buffer (pH 5.2). (A) Normal tRNA; (B) FU-containing tRNA.

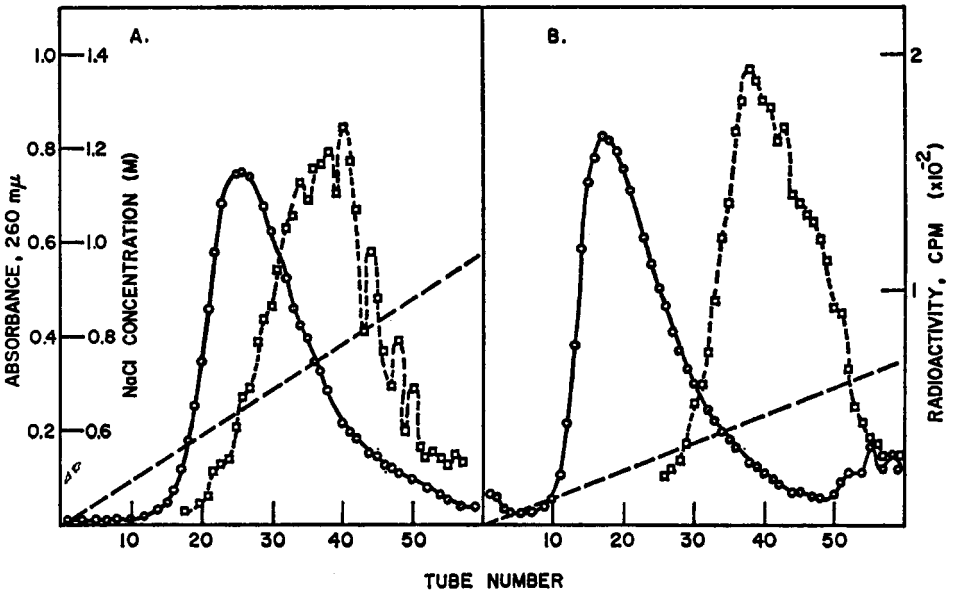

FIGURE 2: Chromatography of mixed normal and [^{14}C]FU-containing tRNA on MASA. *E. coli* exposed to [^{14}C]FU for 2 hr was mixed with a tenfold excess of normal unlabeled cells and tRNA isolated. The RNA was eluted with a linear gradient of NaCl in: (A) 0.02 M sodium acetate buffer (pH 5.2); (B) 0.05 M Tris-HCl buffer (pH 8.0). (○—○) absorbance at 260 mμ; (□—□) radioactivity.

cellulose also failed to detect oligo- or polynucleotide contaminants in our tRNA preparations.

Gel filtration on Sephadex G-100 did not separate FU and normal tRNA. This is demonstrated in Figure 3, which shows the results obtained when FU-tRNA labeled with [^{14}C]FU was chromatographed together with a tenfold excess of unlabeled normal tRNA. The elution pattern of the normal tRNA (A_{260} profile) coincides with that of the ^{14}C-labeled FU-tRNA. A small amount of rRNA was present in the tRNA used in this experiment (peak at tube 5 in Figure 2); this sample had not been as extensively purified as those used for nucleotide analyses and other experiments. The tRNA preparations used for the majority of experiments contained no appreciable contamination detectable by gel filtration (Figure 4).

FU-Containing 5S rRNA. Since no 5S rRNA was detected in tRNA isolated from FU-inhibited cells, either this low molecular weight rRNA is not produced or it is not released from ribsomes, despite the fact that the ribonucleoprotein particles formed by cells exposed to FU are abnormal (Osawa, 1965; Andoh and Chargaff, 1965; Hills and Horowitz, 1966). The second alternative appears to be the correct one, for our results indicate that 5S RNA is formed by FU-treated *E. coli* and the analog is incorporated into this species of RNA. This is

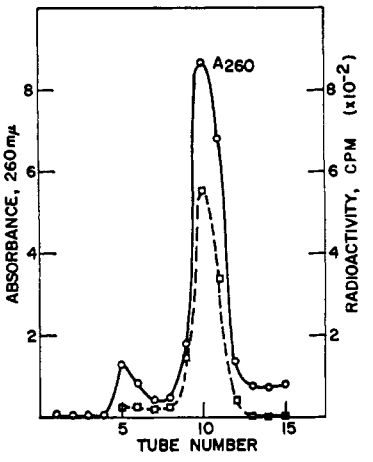

FIGURE 3: Gel filtration of mixed normal and [^{14}C]FU-containing tRNA on Sephadex G-100. *E. coli* exposed to [^{14}C]-fluorouracil for 2 hr was mixed with a tenfold excess of normal unlabeled cells and tRNA prepared as described in Methods. The RNA was eluted with 1 M NaCl; (O—O) absorbance at 260 mμ; (□—□) radioactivity.

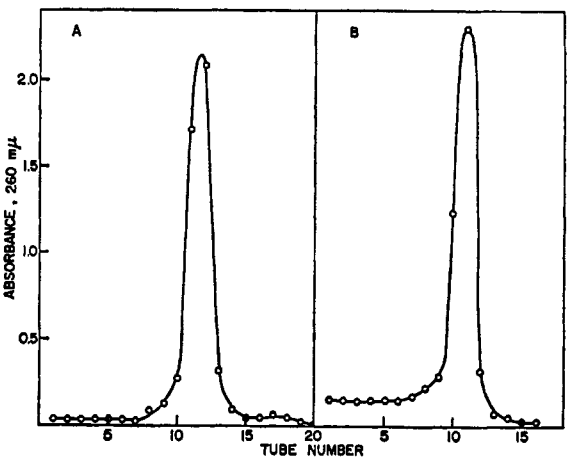

FIGURE 4: Gel filtration of tRNA on Sephadex G-100. RNA was eluted from the column with 1 M NaCl. (A) Normal tRNA; (B) FU-containing tRNA.

demonstrated in Figure 5, which shows the elution pattern obtained when the low molecular weight RNA, prepared from ribosomes isolated from *E. coli* exposed for 2 hr to ^{14}C-labeled FU, was chromatographed on Sephadex G-100. Most of the high molecular weight rRNA was precipitated with 1 M NaCl and the salt-soluble fraction placed on the column. Each of the RNA components contains [^{14}C]FU; the relative specific activities (counts per minute per A_{260} unit) of the peak tubes in the various fractions are: rRNA (tube 12), 0.43; 5S rRNA (tube 17), 0.52; and tRNA (tube 23), 1.

The nucleotide composition of FU-containing 5S rRNA, freed of contaminating polynucleotides by two passages through Sephadex G-100, is shown in Table III together with that of FU-tRNA and FU-rRNA from the same experiment. Other than the substitution of fluorouridylate for uridylic acid, there appears to be no change in the base ratios of 5S RNA or any of the other ribonucleic acids. tRNA had the largest substitution of FU for uracil, while rRNA had the least; the relative extent of replacement of uracil by FU is: rRNA, 0.41; 5S RNA, 0.68; and tRNA, 1.

Amino Acid Acceptor Acitivity. The ability of FU-tRNA to accept amino acids was compared with that of normal tRNA for 19 amino acids (Table IV). It seems evident that FU-tRNA still retains much of its capacity to be charged with amino acids. Of the amino acids tested, 14 were able to acylate FU-tRNA at least as well as normal tRNA, and five, Asp, Asn, Glu, Gln, and Leu, showed a somewhat reduced activity, 70–80% that of normal. While FU-tRNA contains some normal tRNA, present in the cells before they were exposed to FU, the extent to which FU-tRNA is charged clearly indicates that the activity cannot be ascribed only to the normal tRNA present in the preparation. In the experiments shown in Table IV, FU-tRNA was prepared from *E. coli* exposed to FU for 2 hr and had 61% (expt I) and 51% (expt II) of its uracil replaced by FU; the RNA used in expt I had only one-third the normal pseudouridylic acid content. Corrections were not made for isotope dilution of the radioactive amino acids by unlabeled amino acids present in the synthetase preparations. Since different amino acid synthetase preparations were used, this may account for the quantitative differences observed in the extent of charging with certain amino acids in the two experiments shown in Table IV.

Examination of the kinetics of charging normal and FU-tRNA revealed no differences for six amino acids, Phe, Asp, Ala, Leu, Val, and Ile.

tRNA concentration in these experiments was calculated from the absorbance of tRNA solutions at 260 mμ. The spectral characteristics of FU and normal tRNA were identical; the atomic extinction co-

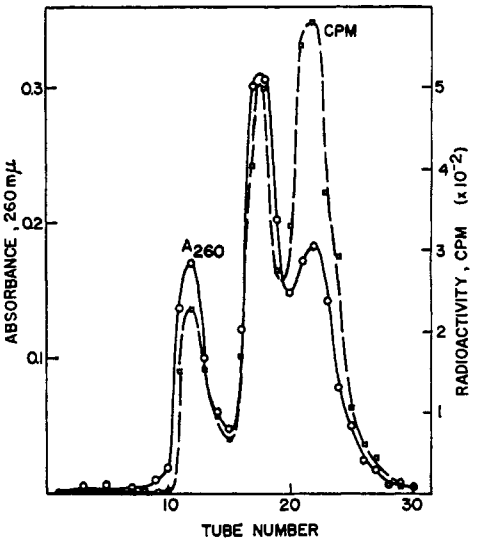

FIGURE 5: Gel filtration on Sephadex G-100 of ribosome-associated low molecular weight RNA from FU-treated *E. coli*. Ribosomes were prepared from *E. coli* exposed to FU for 2 hr and the RNA was isolated by the phenol–sodium dodecyl sulfate procedure (see Methods). The major portion of the high molecular weight rRNA was removed by precipitation with 1 M NaCl before the sample was placed on the column. (O—O) Absorbance at 260 mμ; (□—□) radioactivity.

TABLE III: Nucleotide Composition of r- and tRNAs from FU-Treated *E. coli*.[a]

Type of RNA		Nucleotide Constituent (mole %)						FU × 100
		A	C	G	U	FU	U + FU	U + FU
5S rRNA	Normal[b]	19.2	30.0	33.7	17.1		17.1	
	FU	20.7	30.8	31.7	11.0	5.9	16.9	35
rRNA	Normal[c]	26.4	21.8	30.6	21.1		21.1	
	FU	25.7	22.9	30.9	16.2	4.3	20.5	21
tRNA	Normal	19.9	30.1	31.5	18.5		18.5	
	FU	19.9	28.9	31.5	9.6	10.1	19.7	51

[a] Nucleotides were separated by paper chromatography as described by Lane (1963). Cells were treated with FU (25 μg/ml) for 2 hr and the respective ribonucleic acids were isolated as described under Methods. [b] Nucleotide composition calculated from the known sequence of *E. coli* 5S rRNA (Brownlee *et al.*, 1968). [c] Results from analyses by Andoh and Chargaff (1965).

TABLE IV: Amino Acid Accepting Activity of Normal and FU-Containing tRNA.[a]

	Expt I		Expt II			Expt I		Expt II	
	Normal	FU	Normal	FU		Normal	FU	Normal	FU
Ala			0.025	0.022	Lys	0.036	0.037	0.031	0.036
Arg	0.036	0.038	0.049	0.062	Met			0.041	0.052
Asp			0.037	0.025	Phe	0.013	0.017	0.024	0.029
Asn			0.034	0.026	Pro	0.020	0.012	0.050	0.059
Glu	0.029	0.024	0.030	0.022	Ser	0.018	0.019	0.038	0.040
Gln			0.010	0.006	Thr			0.033	0.034
Gly	0.049	0.054	0.050	0.061	Trp			0.006	0.006
His			0.031	0.031	Tyr	0.010	0.008	0.021	0.019
Ile			0.018	0.017	Val	0.071	0.067	0.059	0.082
Leu			0.089	0.071					

[a] Moles of amino acid per mole of tRNA assuming a molecular weight of 26,500.

efficients with respect to phosphorus, ϵ(P), at 260 mμ were 6800 and 6900, respectively, in 0.01 M Tris-HCl buffer (pH 7.4) containing 5×10^{-4} M magnesium acetate.

Discussion

The results of the present study and our earlier observations (Horowitz and Huntington, 1967; also see Lowrie and Bergquist, 1968) demonstrate that FU alters the composition of *E. coli* tRNA in two respects. First of all, it is incorporated into the molecule, replacing as much as 70% of the uracil with no significant changes in the proportions of the other major nucleotides. Second, there is a marked reduction in the pseudouridylic and ribothymidylic acid content of FU-tRNA (Table I). This decrease is, at least for pseudouridylate, roughly proportional to the extent of FU incorporation (Table II); presumably FU substitutes for the minor base components. It is not surprising that the analog interferes with the formation of these two uracil derivatives since FU is known to inhibit the synthesis of thymidylic acid from deoxyuridylic acid (Cohen *et al.*, 1958), a reaction which also involves substitution on the C-5 of the pyrimidine ring. Earlier evidence indicating that FU and fluoroorotate interfere with pseudouridine synthesis was presented by Wagner and Heidelberger (1962) for a rat liver system, and Robbins and Kinsey (1963) for yeast.

The low levels of pseudouridine and ribothymidine in FU-tRNA offer an opportunity for examining the role of these minor base constituents in the biological function of tRNA. However, any experiments designed to do this will be complicated by the unknown effects of the substitution of FU for uracil and by the presence of normal tRNA along with the FU-containing species in the tRNA isolated from FU-treated cells. Attempts were, therefore, made to separate the two types of RNA. These were at least partially successful in that resolution of FU- and normal tRNA was obtained on columns of MASA (Figure 2), the analog-containing tRNA eluting

after the normal. FU-tRNA is adsorbed more strongly to the anion exchanger, especially as the pH of the eluting buffer is increased, presumably because the lower pK of FU, 8.1–8.3 in poly FU (Szer and Shugar, 1963; Massoulié *et al.*, 1966), compared with that of uracil, 9.6–9.8 in poly U, results in a larger net negative charge on the fluoropyrimidine-containing RNA as the pH approaches 8.0. Use has been made of this effect of higher pH on tRNA containing the analog to fractionate FU-tRNA on DEAE-cellulose (I. I. Kaiser, personal communication, 1968).

The various chromatographic procedures employed failed to detect contamination of the tRNA preparations with rRNA degradation products or 5S rRNA (Figures 1 and 4). There was a possibility that the latter might be released from ribosomes to contaminate the tRNA fraction in FU-inhibited cells since the analog is known to interfere with ribosome formation, resulting in the production of altered ribosomes, the FU particles (Osawa, 1965; Andoh and Chargaff, 1965; Hills and Horowitz, 1966). Our results show clearly that 5S rRNA formed in cells treated with FU continues to be associated with ribonucleoprotein particles and sediments with them in the ultracentrifuge, despite incorporation of the analog (Figure 5). Except for the substitution of FU for uracil, there appears to be no alteration in the nucleotide composition of 5S RNA (Table III). Replacement of uracil by FU is more extensive in tRNA than in 5S RNA, but the analog is more readily incorporated into the latter than into higher molecular weight rRNA.

Since FU-tRNA retains much of its amino acid acceptor capacity despite drastic reductions in the pseudouridylic and ribothymidylic acid content (Table IV; also see Lowrie and Bergquist, 1968), it is likely that these minor base constituents contribute little or nothing to this aspect of tRNA function. Comparison of accepting activity with normal tRNA (Table IV) reveals some differences, but these are relatively minor, and there are as many instances of increases in activity as decreases (Table IV). It is not clear whether the differences observed are due to alterations in the amino acid accepting capacity of a few tRNAs, perhaps because FU is not incorporated to the same extent into all tRNA molecules, or to changes in the relative amounts of the various tRNA species. A comparison of the results in expt I of Table IV with those in expt II provides some support for the latter alternative. An attempt was made to rule out differences due to an absence of the -CpCpA end group of tRNA by including CTP in the reaction mixture in addition to ATP. It should be pointed out that the aminoacylation reactions were carried out under uniform conditions for all the amino acids tested, conditions which were not necessarily optimal for each amino acid. There remains the possibility that larger differences between the activity of normal and FU-tRNA would become evident if each amino acid were tested under optimal conditions.

A number of earlier reports dealing with the activity of FU-tRNA have appeared and while there seems to be some conflict, most results indicate that the fluoropyrimidine-containing tRNA functions well in accepting amino acids. Thus, Gros *et al.* (1962) briefly note that FU-tRNA from *E. coli* can accept arginine, proline, and tyrosine as well as normal tRNA. Their previous results had, however, indicated that the incorporation of FU into tRNA inhibits acceptor activity for proline and tyrosine (Naono and Gros, 1960). The data of Gray and Rachmeler (1967) show a marked inhibition of accepting activity, following incorporation of the analog, for eight amino acids. It is somewhat difficult to interpret their findings, however, because the levels of incorporation they observed were quite low. In yeast, a preliminary account (Ebel *et al.*, 1965) shows that the acceptor activity of FU-tRNA is greater than, equal to, or less than that of normal tRNA, depending upon the amino acid tested.

Examination of FU-tRNA's ability to function in the later stages of protein synthesis may provide some insight into the contribution of the minor bases to the biological function of tRNA and to the possible role of the GpTpψpC sequence, common to all tRNAs (Zamir *et al.*, 1965), which should be replaced by GpFUpFUpC in FU-tRNA. Lowrie and Bergquist (1968) have done some preliminary experiments along these lines, and they noted little difference between normal and FU-tRNA in binding to ribosomes or incorporation of phenylalanine or lysine into polypeptides with poly U or A as synthetic messengers. FU-tRNA, however, failed to transfer lysine into polypeptides when bacteriophage R17 RNA served as messenger; phenylalanine incorporation was also somewhat reduced. The enhancement of incorporation by added tRNA was quite low in their system, and the results should be examined more closely.

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Quantum Yield for the Photoproduced Electron Paramagnetic Resonance Signal in Chromatophores from *Rhodospirillum rubrum**

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ABSTRACT: A 1:1 correspondence has been demonstrated between the concentrations of photoproduced spins in chromatophores of *Rhodospirillum rubrum*, as measured by electron paramagnetic resonance techniques, and the concentration of the reaction center primary electron donor molecule (P_{870} or $P_{0.44}$), as measured by absorbance change at 865 nm. In addition a significantly high quantum yield (0.8 with 880-nm light) has been determined for the electron paramagnetic resonance signal production. When all existing data relating to the photoproduced absorbance change at 865 nm and the photoproduced electron paramagnetic resonance signal are reviewed, together with these results, the conclusion seems inescapable that these are two properties reflecting change in oxidation state of a single molecular species.

From the very first measurements of the photoproduced electron paramagnetic resonance signal in photosynthetic systems (Commoner *et al.*, 1956, 1957; Sogo *et al.*, 1957) it was suggested that the signal arises as a result of photoexcitation of a chlorophyll (or bacteriochlorophyll) molecule at the reaction center. Subsequent experiments in many different laboratories have shown that (a) the location and shape of the signal is consistent with its identification as a porphyrin derivative (Calvin, 1959; Mauzerall, 1968; Mauzerall and Feher, 1964; Mauzerall *et al.*, 1967); (b) the ability to form it at low temperature (Androes *et al.*, 1962) is consistent with its assignment to a primary event; (c) its redox midpoint potential (Loach *et al.*, 1963) and kinetics

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