METABOLISM OF URACIL AND 5-FLUOROURACIL BY DRUG-SENSITIVE AND BY DRUG-RESISTANT BACTERIA

R. W. BROCKMAN, JOAN M. DAVIS AND PATRICIA STUTTS

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala. (U.S.A.)*

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SUMMARY

Escherichia coli resistant to growth inhibition by 5-fluorouracil has lost the capacity to form 5'-ribonucleotide derivatives of uracil and 5-fluorouracil. Enzyme preparations from the resistant *E. coli* cells failed to catalyze the reactions of uracil and 5-fluorouracil with 5-phosphoribosyl-1-pyrophosphate, whereas similar enzyme preparations from the parent drug-sensitive *E. coli* did catalyze these reactions.

These findings suggest that one mechanism of resistance to 5-fluorouracil, at least in the case of the highly resistant bacterial cells studied, is of the same type previously shown to exist for 8-azaguanine and 6-mercaptopurine—namely, failure to form fraudulent ribonucleotide derivatives from the corresponding base.

INTRODUCTION

Presently available evidence shows that a major biosynthetic pathway by which purine bases are converted to ribonucleotides is by reaction with 5-phosphoribosyl-1-pyrophosphate (PRPP)[1,2]. WAY AND PARKS[3,4] have shown that purine analogs, such as 8-azaguanine, 6-mercaptopurine and pyrazolo(3,4-*d*)pyrimidines, react with PRPP in the presence of enzymes from mammalian liver to yield fraudulent purine ribonucleotides. LUKENS AND HERRINGTON[5] have also studied the enzyme-catalyzed reaction of 6-mercaptopurine with PRPP.

In a comparison of the metabolism of purine analogs by actively growing bacterial and neoplastic cells that were sensitive or resistant to 8-azaguanine and 6-mercaptopurine, it was observed that the drug-resistant cells failed to form significant amounts of ribonucleotide derivatives of these analogs as compared to the drug-sensitive cells[6,7]. It has also been demonstrated that enzyme preparations from drug-resistant bacterial and neoplastic cells lack the capacity to catalyze the reaction of certain of the purines and of the relevant purine analogs with PRPP to yield ribonucleotides[8,9]. On the basis of these findings, it was postulated that fraudulent purine ribonucleotides, such as 8-azaguanylic acid and 6-mercaptopurine ribonucleotide or

Abbreviations: U, uracil; UMP, uridine-5'-monophosphate; UDP, uridine-5'-diphosphate; UTP, uridine-5'-triphosphate; UDPG, uridine-5'-diphosphate glucose; UDPAG, uridine-5'-diphosphate-N-acetylglucosamine; 5-fluorouracil analogs are abbreviated as FU, *etc.*; CMP, cytidine-5'-monophosphate; CDP, cytidine-5'-diphosphate; CTP, cytidine-5'-triphosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; Tris, tris(hydroxymethyl)aminomethane–HCl buffer.

* Affiliated with Sloan-Kettering Institute for Cancer Research, New York.

derivatives of these compounds, were active inhibitors and that one mechanism of resistance to these analogs was the deletion or inactivation of enzymes catalyzing the lethal synthesis of these fraudulent ribonucleotides.

It was considered of interest to extend this work to include studies of resistance to pyrimidine analogs. It has been demonstrated that pyrimidine nucleotides are formed from orotidylic acid following the condensation of orotic acid with PRPP[10]. Recently CRAWFORD *et al.*[11] and CANELLAKIS[12] have shown that uridine-5'-phosphate can be formed by direct condensation of uracil with PRPP and that the enzyme catalyzing this reaction is distinct from orotidylic acid pyrophosphorylase[11]. This route of uridylic acid synthesis is distinct from the uracil to uridine[13,14] and uridine to uridylic acid[12] pathway demonstrated in bacterial and mammalian cells and recently investigated by REICHARD AND SKÖLD[15] in ascites tumor cells.

In the light of these findings, we were led to study the metabolism of uracil and 5-fluorouracil in bacteria that are sensitive or resistant to 5-fluorouracil, a compound which possesses interesting anticancer and antibacterial activities[16,17].

METHODS

Materials

The 5-fluoropyrimidine derivatives used in this study were obtained from Hoffmann-La Roche Inc., Nutley, N.J., through the generosity of Dr. ROBERT DUSCHINSKY. [2-^{14}C]Uracil (2 mC/mmole) and 5-[2-^{14}C]fluorouracil (2.5 mC/mmole) were purchased from Isotopes Specialties Company, Burbank, Calif. Pyrimidine nucleosides and nucleotides, UDPG, and the magnesium salt of PRPP were purchased from Pabst Laboratories, Milwaukee, Wisc. A sample of uridine diphosphate N-acetylglucosamine was obtained from Dr. ELIZABETH S. MAXWELL, National Institutes of Health, Bethesda, Md.

Microorganisms

Escherichia coli (American Type Culture Collection 9637) was the parent drug-sensitive organism from which a 5-fluorouracil-resistant strain, designated *E. coli*/FU, was isolated by serial transfer in minimal medium[18] containing increasing concentrations of 5-fluorouracil. The resistant organism used in this study reached maximum growth comparable to that of the parent strain on overnight incubation at 37° in medium containing 5-fluorouracil (1000 μg/ml; 7.6 μmoles/ml). It was observed that growth of the parent *E. coli* 9637 was completely inhibited by 1.0 μg/ml (0.007 μmoles/ml) of 5-fluorouracil.

In the growth inhibition studies with 5-fluoropyrimidines (Table I), solutions of the compounds were sterilized by filtration and added aseptically to tubes of minimal medium[18]. Growth was measured by optical density determinations at 660 mμ in the Bausch and Lomb Spectronic 20 photoelectric colorimeter.

Pyrimidine metabolism

The intermediary metabolism of [2-^{14}C]uracil and 5-[2-^{14}C]fluorouracil was studied in exponentially growing cultures of *E. coli* 9637 and *E. coli*/FU. A standard inoculum was prepared from 18-h cultures of saline-washed cells. The initial optical density of the medium was adjusted to approximately 0.2. The inoculated medium

TABLE I

EFFECT OF 5-FLUOROPYRIMIDINES AND DERIVATIVES ON THE GROWTH OF DRUG-SENSITIVE AND DRUG-RESISTANT *Escherichia coli**

Inhibitor	*Concentration for 50 % inhibition (μmoles/ml)*		*Cross resistance index***
	E. coli *9637*	E. coli/*FU*	
5-Fluorouracil	0.005	> 5.0	> 1000.0
5-Fluorouridine	0.005	> 1.0	> 200.0
5-Fluoro-2′-deoxyuridine	0.006	0.1	16.5
5-Fluorocytosine	2.1	> 5.0	> 2.4
5-Fluorocytidine	0.004	> 1.0	> 250.0
5-Fluoro-2′-deoxycytidine	0.007	1.0	143.0
5-Fluoroörotic acid	0.07	0.8	11.4

* Growth after 16 h incubation at 37° was measured in the Bausch and Lomb Spectronic 20 as optical density at 660 mμ.

** Cross resistance index = $\frac{\text{concentration of inhibitor for 50 \% inhibition of } E.\ coli/\text{FU}}{\text{concentration of inhibitor for 50 \% inhibition of } E.\ coli\ 9637}$.

was then divided into 65-ml portions in 300 ml Erlenmeyer flasks fitted with 18 × 160 mm culture tubes by means of a ground-glass joint. The flasks, which were mechanically agitated in a water bath at 37°, could be inverted and the optical density of the medium conveniently determined at intervals in order that growth curves could be plotted. The incubation flasks were fitted with sidearms to permit convenient addition of radioactive substrates and to admit air through sterile cotton plugs. After 120 min incubation of the cultures, the optical density approached 0.7 and radioactive substrates were added; maximum growth of control cultures (optical density of approximately 1.0) was reached about 180 min after inoculation under these conditions. The generation times of *E. coli* 9637 and of *E. coli*/FU were 50 min and 54 min, respectively, under these experimental conditions. 15 Min after the radioactive substrates were added, growth was stopped by pouring the cultures on 35 g of ice. Cells were removed by centrifugation and were then re-suspended in 10 ml of ice water. The cell suspension was immediately poured into 40 ml of boiling ethanol and extracted for 5 min. Cell debris was removed by centrifugation; the clear supernatant solution thus obtained was concentrated to a small volume by evaporation under reduced pressure and was then lyophilized. The residue was dissolved in approximately 0.5 ml of water; the exact volume of water used was proportional to the optical density of the culture when it was poured on ice. In this manner comparable cell extracts were prepared for paper chromatography. The chromatographic-radioautographic analysis of such extracts has been described[7,19].

A study of the incorporation of [2-^{14}C]uracil and of 5-[2-^{14}C]fluorouracil into the nucleic acids of *E. coli* 9637 and of *E. coli*/FU was made. To 500 ml cultures of bacteria incubated at 37° in a shaking incubator was added 8.75 μC of radioactive substrate. When maximum growth was reached approximately 4 h later the cells were separated by centrifugation and extracted with hot aqueous ethanol as described above. The cell debris served as the starting point for the isolation of the nucleic acid by procedures previously described[7]. Specific activities (μC/100 μg) of the combined nucleic acid (RNA + DNA) thus obtained were determined by evaporating solutions of nucleic acid on planchets and counting in gas flow proportional counters (Table II).

TABLE II

INCORPORATION OF [2-^{14}C]URACIL AND 5-[2-^{14}C]FLUOROURACIL INTO THE NUCLEIC ACID OF *Escherichia coli*

Combined nucleic acids from:*	*Specific activity of nucleic acid** (counts/sec/100 μg)*	
	[2-^{14}C]Uracil	*5-[2-^{14}C]Fluorouracil*
E. coli 9637	348	287
E. coli/FU	< 1	< 1

* Combined nucleic acids (CNA) = RNA + DNA.

** Cultures of *E. coli* were incubated in the presence of the radioactive substrates for approximately 4 h. Alkaline hydrolysis of CNA followed by electrophoretic analysis of ribonucleotides demonstrated the presence in RNA of uridylic and cytidylic acids derived from [2-^{14}C]uracil and of 5-fluorouridylic acid derived from 5-[2-^{14}C]fluorouracil.

Alkaline hydrolysates of nucleic acid were analyzed by paper electrophoresis in ammonium formate buffer (pH 3.5); radioactive pyrimidine ribonucleotides were detected by exposing the electrophoresis strips to X-ray film.

Enzyme preparations

Soluble enzyme preparations were made from cells harvested by centrifugation from 500-ml cultures of *E. coli* 9637 and *E. coli*/FU grown for 18 h at 37°; aeration of the cultures was provided by mechanical agitation. The cells were washed twice in physiological saline, suspended in 10 ml of 0.2 *M* Tris buffer (pH 7.6) and then subjected to sonic vibration* at 0° for 5 min. The supernatant solution obtained after centrifugation of the resulting suspension at 25,000 × *g* for 1 h at 4° served as the soluble enzyme preparation. Protein content of such enzyme preparations was determined by the OYAMA-EAGLE modification[20] of the method of LOWRY *et al.*[21] using crystalline bovine albumin (Armour and Co.) as a reference standard**. Similar enzyme preparations in 0.1 *M* phosphate buffer (pH 7.2) were also made; these were comparable in activity to the Tris buffer preparations. After reaction of pyrimidines or purines with PRPP in the presence of the enzyme preparations the incubation medium was analyzed by paper chromatography to separate the pyrimidine or purine bases from the nucleotides. The % conversion of base to nucleotide was determined by eluting the radioactive compounds, evaporating the eluates on steel planchets, and quantitatively measuring the activity in internal gas-flow proportional counters.

RESULTS

Response of E. coli *9637 and* E. coli*/FU to 5-fluoropyrimidine derivatives*

It was observed that 5-fluorouracil, 5-fluorouridine, 5-fluoro-2′-deoxyuridine, 5-fluorocytidine, and 5-fluoro-2′-deoxycytidine were completely inhibitory to the growth of *E. coli* 9637 at low concentrations (Table I). Higher levels of 5-fluoroörotic

* Raytheon 9-kc, 50-W Magnetostriction Oscillator, Raytheon Corp., Waltham, Mass.

** A Tris buffer blank was used in the protein determinations to compensate for the color developed by Tris with the reagents used.

acid were required for complete inhibition; 5-fluorocytosine was a poor growth inhibitor. Growth of the 5-fluorouracil-resistant organism (*E. coli*/FU) was inhibited by the other 5-fluoropyrimidine compounds at concentrations higher than those required to inhibit *E. coli* 9637 (Table I). It is of interest that 5-fluoro-2′-deoxyuridine proved to be the most effective inhibitor of *E. coli*/FU in these experiments.

Metabolism of [2-^{14}C]uracil and 5-[2-^{14}C]fluorouracil by sensitive and 5-fluorouracil-resistant microörganisms

It was found that [2-^{14}C]uracil was extensively metabolized to nucleotide derivatives in 15 min by exponentially growing cultures of the parent drug-sensitive *E. coli* 9637 but not by *E. coli*/FU (Fig. 1). Similarly, 5-[2-^{14}C]fluorouracil was metabolized to nucleotide derivatives by sensitive but not by resistant cells (Fig. 1). It was also observed that *E. coli* 9637 incorporated significant amounts of [2-^{14}C]-uracil and of 5-[2-^{14}C]fluorouracil into its nucleic acid (Table II). Incorporation into RNA of [2-^{14}C]uracil as uridylic and cytidylic acids and of 5-[2-^{14}C]fluorouracil as 5-fluorouridylic acid was shown by electrophoretic analysis of alkaline hydrolysates of nucleic acid samples. No 5-fluorocytidylic acid could be detected. There was no significant incorporation of uracil or of 5-fluorouracil into the nucleic acids of *E. coli*/FU (Table II).

A comparison of the metabolism of [2-^{14}C]uracil and 5-[2-^{14}C]fluorouracil by exponentially growing *Streptococcus faecalis* that are sensitive (SF/O), or resistant (SF/FU), to growth inhibition by 5-fluorouracil gave similar results. Thus, [2-^{14}C]-uracil and 5-[2-^{14}C]fluorouracil were extensively metabolized to nucleotides by SF/O but were not converted to nucleotide derivatives by SF/FU*. These results are analogous to those obtained in the study of resistance to 8-azaguanine and 6-mercaptopurine[7].

The pyrimidine nucleotides were not adequately resolved by the two-dimensional chromatographic system used (phenol–water : *n*-butanol–propionic acid–water). However, one-dimensional chromatography of *E. coli* 9637 extracts in isobutyric acid–ammonium isobutyrate[22] or in ethanol–ammonium acetate[23,24] showed a number of separate radioactive spots derived from either [2-^{14}C]uracil or 5-[2-^{14}C]-fluorouracil. These derivatives behaved chromatographically like uracil, UMP, UDP, UTP, UDPG and UDPX, where X is probably N-acetylglucosamine (Fig. 2). In all cases the 5-fluorouracil derivatives behaved chromatographically like those of uracil. Further evidence for the identity of the [2-^{14}C]uracil and 5-[2-^{14}C]fluorouracil derivatives was obtained by chemical and enzymic hydrolysis of the nucleotides. Hydrolysis by 1 *N* hydrochloric acid at 90° for 30 min converted the di- and triphosphate derivatives (including the nucleoside diphosphate glycosyl derivatives) to compounds chromatographically identical with uridylic acid and 5-fluorouridylic acid. Intestinal phosphatase hydrolyzed uridiylic and 5-fluorouridylic acids to the corresponding nucleosides. The radioactive nucleoside derived from UMP was identified as [2-^{14}C]uridine by co-chromatography with uridine in several solvent systems and by co-electrophoresis with uridine in borate buffer. Similarly, 5-fluorouridine was identified as the radioactive nucleoside derived from FUMP.

The compounds derived from [2-^{14}C]uracil and 5-[2-^{14}C]fluorouracil which

* Unpublished observations, C. SPARKS, P. STUTTS and R. W. BROCKMAN, Southern Research Institute.

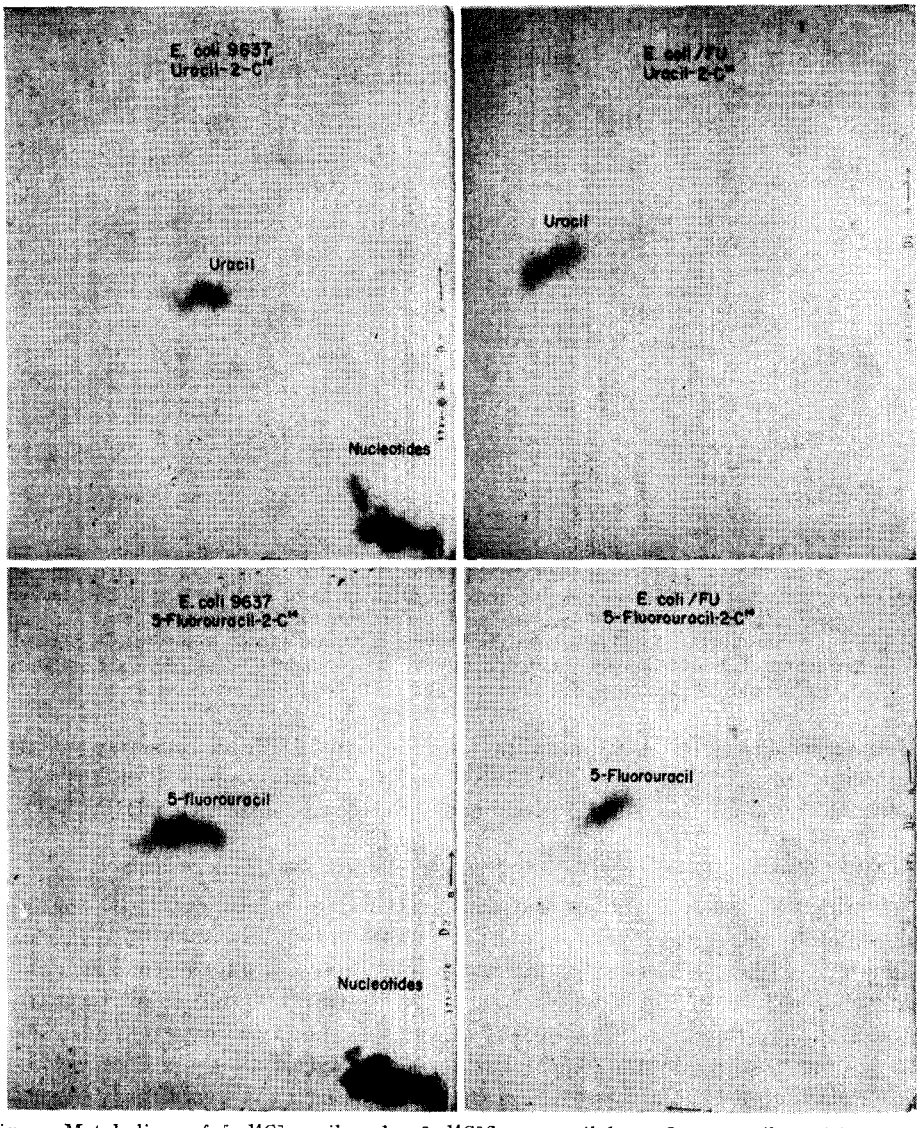


Fig. 1. Metabolism of [2-^{14}C]uracil and 5-[2-^{14}C]fluorouracil by 5-fluorouracil-sensitive (9637) and -resistant (FU) *Escherichia coli*. Extracts of cells were analyzed by paper chromatography in two dimensions: phenol–water (←) followed by *n*-butanol–propionic acid–water (↑). Chromatograms were exposed for 2 weeks to X-ray film (Eastman Kodak single-emulsion, blue-sensitive, 14 × 17″) to detect radioactive compounds.

migrated like UDPG were eluted from the one-dimensional chromatogram developed in ethanol–ammonium acetate (Fig. 2). Both of these radioactive derivatives migrated with a known sample of UDPG upon co-chromatography in ethanol–ammonium acetate.

The radioactive "UDPX" and "FUDPX" derivatives migrating just ahead of UDPG upon chromatography in ethanol–ammonium acetate (Fig. 2) are probably N-acetylglucosamine derivatives. LELOIR *et al.*[23,24] reported that uridine diphosphate

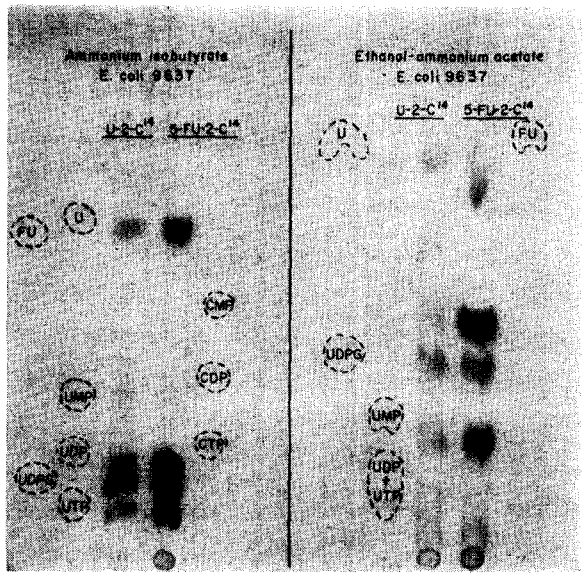


Fig. 2. Chromatographic-radioautographic analysis of extracts of *E. coli* 9637 incubated with [2-^{14}C]uracil (U-2-C^{14}) or 5-[2-^{14}C]fluorouracil (5-FU-2-C^{14}). One-dimensional descending chromatography using Whatman No. 3 MM paper with isobutyric acid–ammonium isobutyrate[22] or ethanol–ammonium acetate[23] resolved nucleotides and uridine diphosphate glycosyl derivatives. Dotted areas indicate the positions of u.v.-absorbing reference compounds.

N-acetylglucosamine (UDPAG) migrates just ahead of UDPG in this solvent system. The identity of "UDPX" and "FUDPX" as N-acetylglucosamine derivatives was confirmed by chromatography of these radioactive compounds with UDPAG.

A physicochemical effect of 5-fluoro-substitution on uracil and its derivatives

During the course of work on the identification of metabolic derivatives of uracil and 5-fluorouracil, it was observed that the 5-fluoro derivatives of orotic acid, uracil, uridine and 2′-deoxyuridine migrated significantly more rapidly upon high voltage electrophoresis[25] than did the corresponding unsubstituted pyrimidine derivatives (Table III). The fluorinated derivatives of cytosine, however, migrated identically with the unsubstituted compounds. These results suggest that the dissociation of the hydroxyl group in the 4-position of the pyrimidine ring is significantly increased as a result of the influence of the fluorine substituent in the 5-position. CHAUDHURI, MONTAG AND HEIDELBERGER[26] observed that 5-fluoro-substituted pyrimidines were more acidic than the corresponding pyrimidines upon ion-exchange chromatography. It is also of interest that GINER-SOROLLA AND BENDICH[27] observed a marked increase in the acid strength of 6-methyluracil upon replacement of the hydrogen atoms of the methyl group by fluorine atoms. DAHL, WAY AND PARKS[28] recently observed that orotic and 5-fluoroörotic acids have similar Michaelis constants when compared as substrates for yeast orotidylic acid pyrophosphorylase. These authors also observed that the pH optima for nucleotide formation are similar and that 5-fluoroörotic acid reacts about twice as rapidly as does orotic acid. The substitution of the fluorine atom on the pyrimidine ring alters both the physico-chemical properties of the molecule and the rate of an enzyme catalyzed reaction.

TABLE III

ELECTROPHORETIC MIGRATION OF PYRIMIDINE DERIVATIVES: EFFECT OF 5-FLUORO-SUBSTITUTION*

	*Relative migration distance of pyrimidine derivatives***	
	Unsubstituted compound	*5-Fluoro-substituted compound*
Uracil	30	82
Uridine	66	85
2′-Deoxyuridine	18	60
Cytosine	— 5	— 4
Cytidine	46	42
2′-Deoxycytidine	—12	—12
Orotic acid	97	113

* Electrophoresis on Whatman No. 3 MM paper was carried out in apparatus similar to that described by MARKHAM[25]. Sodium tetraborate (0.05 *M*; pH 9) served as the buffer.

** Relative migration distance $= \frac{\text{migration distance (cm) of pyrimidine}}{\text{migration distance (cm) of uridine-5′-phosphate}} \times 100$

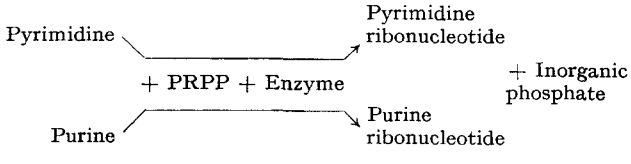
Uridine-5′-phosphate generally moved 14 to 16 cm from the origin upon electrophoresis for 90 min at 750 V. A negative sign indicates migration toward the cathode. The difference in migration of the ribo- and deoxyribonucleosides is apparently due to the formation by the former of an ionized complex with borate through the 2′,3′-*cis*-glycol structure[29].

Electrophoretic analysis of pyrimidine nucleoside derivatives

The electrophoretic mobilities of the pyrimidine compounds and the 5-fluoro-derivatives are given in Table II. The separation of ribo- and deoxyribonucleosides[29] is apparent and an effort was made to detect the presence of 5-fluoro-2′-deoxyuridine derivatives by this means. All of the nucleotide derivatives eluted from the two-dimensional chromatograms (Fig. 1) were completely hydrolyzed to nucleoside derivatives upon incubation overnight at 37° with snake venom (*Crotalus atrox*)*. The nucleosides thus formed were subjected to paper electrophoresis in borate buffer. All of the radioactivity from 5-[2-^{14}C]fluorouracil was contained in 5-fluorouridine; most of the radioactive compounds derived from [2-^{14}C]uracil were present as uridine after snake venom hydrolysis. No radioactive deoxyribonucleosides could be detected; no conversion of fluorinated pyrimidine derivatives to the corresponding pyrimidine derivatives was observed. It is possible that the solvents used in two-dimensional chromatography may have destroyed deoxyribonucleoside derivatives or that the concentrations of such derivatives formed during the 15-min incubation of radioactive substrates with exponentially growing *E. coli* 9637 were too low to be detected.

Studies with enzyme preparations from drug-sensitive and -resistant E. coli

The capacity of enzyme preparations from *E. coli* 9637 and *E. coli*/FU to catalyze the following reactions was studied:



The data summarized in Table IV show that uracil, 5-fluorouracil, orotic acid, and 5-fluoroörotic acid react with PRPP in the presence of enzyme preparations from

* Obtained from Ross Allen's Reptile Institute, Silver Springs, Florida.

E. coli 9637 to afford the corresponding ribonucleotides. Enzyme preparations from *E. coli*/FU failed to catalyze the formation of uridylic and 5-fluorouridylic acids, whereas orotidylic and 5-fluoroörotidylic acids were formed in good yields. The conversion of purines to ribonucleotides, included as a reference reaction, took place equally well in the presence of enzyme preparations from *E. coli* 9637 or *E. coli*/FU.

TABLE IV

FORMATION OF RIBONUCLEOTIDES BY REACTION OF PYRIMIDINE AND PURINE BASES WITH 5-PHOSPHORIBOSYL-1-PYROPHOSPHATE IN THE PRESENCE OF ENZYMES FROM *E. coli* 9637 AND *E. coli*/FU

Biochemical reaction	*Base + PRPP $\xrightarrow{Enzyme}$ Ribonucleotide** % conversion of base to nucleotide*	
	E. coli 9637	E. coli/FU
Uracil ——→ UMP	11.7**	0
5-Fluorouracil ——→ 5-FUMP	12.6**	0
Orotic acid ——→ OMP	53.3**	62.1
5-Fluoroörotic acid → 5-FOMP	68.7**	92.6
Adenine ——→ AMP	33.1	36.3
Guanine ——→ GMP	40.4	47.6
Hypoxanthine ——→ IMP	55.3	78.7
Xanthine ——→ XMP	40.1	47.1

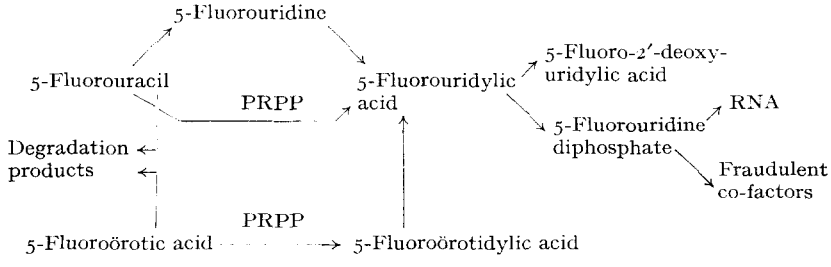
* Experimental conditions: Base (0.5 μmole; 0.2 μC); PRPP (1.0 μmole); enzyme equivalent to approximately 0.3 mg protein; 0.1 *M* Tris buffer (pH 7.6) to a volume of 1 ml. The mixture was incubated for 1 h at 37°.

** These figures are average values from four or more independent experiments. Other data are based on results of assay of the catalytic activity of at least two different enzyme preparations.

DISCUSSION

While the present study was in progress, REICHARD AND SKÖLD[30] described the results of a comparison of the metabolism of uracil in sensitive and 5-fluorouracil-resistant Ehrlich ascites tumor cells. It was demonstrated that the 5-fluorouracil-resistant Ehrlich ascites cells were unable to form 5'-ribonucleotide derivatives of uracil as a result of loss of uridine phosphorylase activity. Uridine kinase activity in the resistant cell line, although lowered, was significant. These results are consistent with the findings of HANDSCHUMACHER[31] recently reviewed by WELCH[32].

From these results, it appears that resistance to 5-fluorouracil can arise by deletion (or inactivation) of uridylic acid pyrophosphorylase, as in the case of *E. coli* in the present study, or by loss of uridine phosphorylase activity as in the case of Ehrlich ascites tumor cells[30]. It appears that 5-fluorouracil is metabolized by the same biosynthetic pathways as is uracil[17,33]:



In studies with 5-fluorouracil, HEIDELBERGER *et al.*[16,34] and EIDINOFF *et al.*[35] observed inhibition of the conversion of formate to the methyl group of thymine. COHEN *et al.*[17] have shown that 5-fluoro-2′-deoxyuridylic acid specifically inhibits the synthesis of thymidylic acid in a strain of *E. coli*, thus causing unbalanced growth. It is therefore understandable that failure to form 5-fluorouridylic acid and hence 5-fluoro-2′-deoxyuridylic acid from 5-fluorouracil would be an effective mechanism of resistance. In view of the multiple steps involved in uracil metabolism, multiple sites of inhibition by pyrimidine analogs[17,33–40] and multiple mechanisms of resistance[41] to such analogs are possible.

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STUDIES ON A PROTEIN-SYNTHESIS-AFFECTING SUBSTANCE FROM BIOLOGICAL MATERIALS

JAN HRADEC AND ALEXANDRA ŠTROUFOVÁ
Department of Biochemistry, Oncological Institute, Prague (Czechoslovakia)

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SUMMARY

A substance of lipid character, containing carbohydrate and phosphorus, which stimulates the incorporation of labelled amino acids in the protein of Ehrlich ascites cells, was found in animal tissues and egg yolk. The content of the tissues of tumour-bearing individuals was higher than that of the tissues of normal ones. The substance was obtained in crystalline form.

INTRODUCTION

In a recent paper the effect was described of homogenates of various rat tissues on the incorporation of labelled methionine into the proteins of Ehrlich ascites cells *in vitro*[1]. It was found that these preparations exhibit a stimulatory as well as an inhibitory effect on this process, depending on the dose used. It seemed very probable that some substance present in the tissues might be responsible for these effects.

Some indications do already exist that there may be factors in tissues capable of influencing the biosynthesis of proteins[2]. Evidence was obtained[1] that tissues of tumour-bearing animals contain higher amounts of the active substance than those of normal ones. It seems quite reasonable to suppose that these different quantities of a protein-synthesis-affecting material in various tissues may be connected in some way with the varying abilities of these tissues to incorporate labelled amino acids *in vitro*. Such differences are known to exist between tumours and corresponding