# 5-Fluorouracil and Derivatives in Cancer Chemotherapy II: Possible *In Vivo* Formation and Stabilization of 5-Fluoro-2′-deoxyuridine

**Keyphrases** □ 5-Fluorouracil—*in vivo* conversion to 5-fluoro-2′-deoxyuridine in presence of nucleosides □ 5-Fluoro-2′-deoxyuridine—*in vivo* formation from 5-fluorouracil in presence of nucleosides, stabilization □ Cancer chemotherapy—*in vivo* formation and stabilization of 5-fluoro-2′-deoxyuridine

*Sir:*

Following the synthesis of 5-fluoro-2′-deoxyuridine by Duschinsky *et al.* (1), it was shown that the nucleoside was approximately $10^3$ times as effective as 5-fluorouracil *in vitro* (2). Ansfield and Curreri (3) suggested that this drug has profound advantages in the treatment of breast and colon cancer. However, the relatively high effectiveness of 5-fluoro-2′-deoxyuridine in respect to 5-fluorouracil has not been borne out in further *in vivo* systems. This probably can be attributed to the fact that 5-fluoro-2′-deoxyuridine, like other pyrimidine nucleosides, cleaves and gives rise to the pyrimidine base and the sugar moiety (4). This apparent instability *in vivo* has been a serious problem in the utilization of this drug in cancer chemotherapy.

We have become interested in conducting biopharmaceutical and pharmacokinetic studies on 5-fluorouracil as well as its derivatives such as 5-fluoro-2′-deoxyuridine. Preliminary work for this study has involved the development of analytical procedures for 5-fluorouracil (5) and 5-fluoro-2′-deoxyuridine. The work dealing with the analysis of 5-fluoro-2′-deoxyuridine revealed that the instability or cleavage of the drug observed in *in vivo* systems continues in blood samples *in vitro* to the extent that approximately 75% of the drug is destroyed in 24 hr. Obviously, any analytical method must be capable of determining the level of the drug present at the time samples are withdrawn from the patient. Therefore, it was necessary to stabilize the 5-fluoro-2′-deoxyuridine.

**Table I**—Stability of 5-Fluoro-2′-deoxyuridine in the Presence of Other Nucleosides under Dialysis Conditions in Whole Human Blood

| Combination | Initial 5-Fluoro-2′-deoxyuridine, *M* | Initial (Other Nucleoside), *M* | 5-Fluoro-2′-deoxyuridine Found after 24 hr., % |
|---|---|---|---|
| 5-Fluoro-2′-deoxyuridine + 5-trifluoromethyl-2′-deoxyuridine | $2.14 \times 10^{-4}$ | $2.03 \times 10^{-3}$ | 82.6 |
| 5-Fluoro-2′-deoxyuridine + deoxyuridine | $2.07 \times 10^{-4}$ | $2.63 \times 10^{-3}$ | 58.6 |
| 5-Fluoro-2′-deoxyuridine | $4.14 \times 10^{-5}$ | — | 25.1 |

**Table II**—5-Fluoro-2′-deoxyuridine Derived from 5-Fluorouracil in the Presence of Various Nucleosides[a]

| Combination | Initial Concentration of Nucleoside, *M* | 5-Fluoro-2′-deoxyuridine Found, *M* | Percent Conversion after 24 hr. |
|---|---|---|---|
| 5-Fluorouracil + deoxycytidine | $2.00 \times 10^{-3}$ | $1.38 \times 10^{-4}$ | 35.8 |
| 5-Fluorouracil + 7-trifluoromethyl-2′-deoxyuridine | $2.00 \times 10^{-3}$ | $1.99 \times 10^{-4}$ | 51.7 |
| 5-Fluorouracil + deoxyuridine | $2.44 \times 10^{-3}$ | $2.44 \times 10^{-4}$ | 63.4 |

[a] Initial [5-fluorouracil] = $3.85 \times 10^{-4}$ *M* under dialysis conditions in whole human blood.

A survey of the literature indicated that others had made this attempt utilizing two approaches: (*a*) the introduction of methylated 5-fluoro-2′-deoxyuridine derivatives (6), and (*b*) the use of substances believed to inhibit nucleoside phosphorylase (7, 8).

It has been reported that compounds such as 5-trifluoromethyl-2′-deoxyuridine were reasonably successful. Some simple dialysis studies were carried out in which human blood was placed in a dialysis cell, as in our previous report (5). Samples of 5-fluorouracil and 5-fluoro-2′-deoxyuridine were each dialyzed under conditions of added nucleosides. The results, as shown in Tables I and II, indicate two findings:

1. The cleavage of 5-fluoro-2′-deoxyuridine is inhibited.
2. 5-Fluoro-2′-deoxyuridine is formed from 5-fluorouracil and the nucleosides.

These findings, which we feel are highly significant, tend to suggest that the previous so-called inhibition of nucleoside phosphorylase may to some part be due to a reversible reaction which leads to the formation of 5-fluoro-2′-deoxyuridine. It is also significant that this reaction did not take place in simple aqueous buffer solutions but must be catalyzed by some blood components.

The impact of this finding on the clinical use of 5-fluorouracil and 5-fluoro-2′-deoxyuridine has led us to investigate this reaction further. It appears that the reaction of Scheme I might to some extent explain our findings and also be the basis to explain some of the rather anomalous findings of other investigators (9).

5-fluorouracil + pyrimidine base·deoxyuridine ⇌ 5-fluoro-2′-deoxyuridine + pyrimidine base

*Scheme I*

Each sample of blood containing the drug(s) was dialyzed against a pH 7.4 phosphate buffer for 24 hr.

After this period, a known volume of the dialyzate was evaporated to dryness, dissolved in a known volume of absolute ethanol, and spotted on a fluorescent thin-layer plate. Following multiple-pass, discontinuous developing in a developer consisting of ethyl acetate–absolute alcohol–10% aqueous ammonia (200:5:2), the air-dried plates were scanned using a thin-film scanning attachment for a spectrophotofluorometer[1]. An activation wavelength of 280 nm. and a fluorescent wavelength of 530 nm. were used. The scan was quantitated by comparing areas of unknowns to those of known standard compounds.

The data show that the exchange reaction did take place for all the nucleosides tried. Furthermore, for optimum conversion of 5-fluorouracil to 5-fluoro-2′-deoxyuridine, an excess of the nucleoside is necessary.

The implication for cancer chemotherapy is that one can, by using an excess of a relatively inexpensive, possibly nontoxic substance like deoxyuridine, obtain within the body at desired levels the more effective 5-fluoro-2′-deoxyuridine from 5-fluorouracil. Obtaining 5-fluoro-2′-deoxyuridine this way may provide an antitumor agent for such forms of cancer as rectal cancer, for which 5-fluoro-2′-deoxyuridine seems by far superior to 5-fluorouracil. The hope that such a conversion may in fact take place is supported by the results of the extensive study of inhibitors referred to earlier in this report (4). In light of the present results, Birnie and Heidelberger's observation of great toxicity in the use of uridine with 5-fluoro-2′-deoxyuridine may be explained in terms of the exchange reaction, where one of the products is the very toxic 5-fluorouridine. As an extension of this, it may be suggested that the effect which has up to now been attributed to inhibition of the nucleoside phosphorylase may be due to a contribution from the exchange reaction noted earlier.

Experiments to test this speculation of the increased efficacy of 5-fluorouracil and 5-fluoro-2′-deoxyuridine are now underway in mice[2]. Preliminary data on both solid and liquid tumors appear to bear out the initial findings. These results will be published.


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[1] Aminco-Bowman.

[2] Collaborative studies now being carried out in the laboratory of Dr. C. Heidelberger, University of Wisconsin.


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# Conformational Stabilization by *o*-Methyl Groups of a Sulfanilamide


**Keyphrases** ☐ Sulfanilamides—conformational stabilization by *o*-methyl groups, NMR, PMR spectroscopy ☐ Conformation, sulfanilamides—NMR evidence for stabilization by *o*-methyl groups ☐ NMR spectroscopy—stabilization of sulfanilamides ☐ PMR spectroscopy—stabilization of sulfanilamides


*Sir:*

The conformational attributes of drug molecules are frequently alluded to in order to account for variations in drug response among members of a noncongeneric or pseudocongeneric series. Conformationally distinct molecules might be expected to differ in their physical properties, *e.g.*, partition coefficient (1) or pKa (2), and in this way differences between the magnitudes of their biological responses could be "explained." Alternatively, the conformational distinction between molecules could be translated at the receptor level as providing differing degrees of stimulus which, in turn, become elicited as variations in the relative responses. The relative importance of each of these alternatives most probably will have to be established for each drug system of interest, but studies having this intent are notably lacking in the literature. In view of recent progress in the study of sulfonamide action (3, 4), it seems appropriate to point out certain of our experimental findings which have significance in relation to the conformational attributes of sulfanilamides in solution.

**Table I**—Calculated Chemical Shifts for the Sulfanilyl Ring Protons of Some $N^1$-Phenylsulfanilamides

| Anilyl Ring Substituent | Absorbance of $N^4$-Acetyl Derivative | $\bar{\nu}$, Hz.[a] | Jobs, Hz. | *Ortho* $\delta_A$, Hz. | *Meta* $\delta_B$, Hz. |
|---|---|---|---|---|---|
| 2-$CH_3O$ | S | 45.8 | 8.8 | 18.0 | 73.6 |
| 2-$CH_3$; 6-$CH_3$ | Q | 47.5 | 8.8 | 23.6 | 71.4 |
| 2-$CH_3$ | Q | 46.7 | 9.0 | 21.9 | 71.6 |
| 2-Cl | S | 44.2 | 8.9 | 16.6 | 71.9 |
| 2-Br | S | 44.8 | 8.8 | 17.2 | 72.4 |
| 2-I | S | 44.0 | 8.9 | 16.8 | 71.2 |
| 2-$NO_2$ | S | 39.5 | 9.0 | 9.7 | 69.4 |

[a] Chemical shift measured to center of pattern relative to the sulfanilyl ring absorption of $N^4$-acetylsulfanilamide (singlet, $\tau$ 2.30).