Biochemical Pharmacology, Vol 31, No. 6, pp. 1103–1108, 1982.
Printed in Great Britain

0006-2952/82/061103-06 $3.00/0

INCORPORATION OF METABOLITES OF 2′-FLUORO-5-IODO-1-β-D-ARABINOFURANOSYLCYTOSINE INTO DEOXYRIBONUCLEIC ACID OF NEOPLASTIC AND NORMAL MAMMALIAN TISSUES

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(*Received* 3 *July* 1981; *accepted* 4 *September* 1981)

Abstract—The radioactivity of ^{14}C-labeled 2′-fluoro-5-iodo-1-β-D-arabinofuranosylcytosine ([2-^{14}C]-FIAC), a new and potent antiherpetic agent, was shown previously to be incorporated into the DNA fractions of mammalian and neoplastic tissues. The present work was undertaken to learn the nature of the incorporated moieties. Enzyme degradation of highly purified DNA from the small intestine of mice treated with [2-^{14}C]FIAC and analysis of the resulting nucleosides failed to reveal the presence of unchanged FIAC. Rather, three metabolites were found, namely, the 2′-fluoro-1-β-D-arabinofuranosyl nucleosides of cytosine (FAC), thymine (FMAU) and 5-iodouracil (FIAU). Labeled metabolites of FIAC were also found in the DNA isolated from P815 leukemic cells in mice given [2-^{14}C]FIAC. It is of interest to note that FMAU, FIAU and FAC are, like FIAC, potent antiherpetic agents.

2′-Fluoro-5-iodo-1-β-D-arabinofuranosylcytosine (FIAC‡) was synthesized recently and found to be a potent agent against herpes simplex virus, types 1 and 2, *in vitro* [1, 2]. Previous work in this laboratory established that in mice given [2-^{14}C]FIAC radioactivity is incorporated into the DNA of small intestine and other organs [3–5]. The present studies deal with the nature of the radioactive moieties that appear in DNA. Since our earlier work had shown that FIAC is deaminated in mice into FIAU and that FMAU is also formed by deamination of FIAC and replacement of the 5-iodo by a 5-methyl group, we anticipated that the labeled DNA moieties might be FIAC itself, FIAU, or FMAU. We were also interested in determining the nature of the labeling of DNA in murine P815 leukemic cells, treated *in vitro* with [2-^{14}C]FIAC, particularly since FIAU and FMAU are more cytotoxic in such cells than FIAC [1]. Finally, we determined the influence of the pyrimidine nucleoside deaminase inhibitor, THU, on the incorporation of the radioactivity of FIAC into DNA in both mice and P815 cells.

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‡ Abbreviations: ara-C, 1-β-D-arabinofuranosylcytosine; ara-U, 1-β-D-arabinofuranosyluracil; FIAC, 2′-fluoro-5-iodo-ara-C; FIAU, 2′-fluoro-5-iodo-ara-U; FAC, 2′-fluoro-ara-C; FAU, 2′-fluoro-ara-U; FMAC, 2′-fluoro-1-β-D-arabinofuranosyl-5-methylcytosine; FMAU, 2′-fluoro-1-β-D-arabinofuranosyl-5-methyluracil; FIACMP, 5′-monophosphate of FIAC; IdCyd, 2′-deoxy-5-iodocytidine; HPLC, high performance liquid chromatography; IdUrd, 2′-deoxy-5-iodouridine; BdCyd, 2′-deoxy-5-bromocytidine; and THU, 3,4,5,6-tetrahydrouridine.

MATERIALS AND METHODS

Chemicals

Aqueous phenol solution (88%), ammonium acetate and potassium hydroxide (pellets) were purchased from the Fisher Scientific Co., Springfield, NJ. Cesium chloride was obtained from Schwarz-Mann, Orangeburg, NY and was of optical grade. 3,4,5,6-Tetrahydrouridine (NSC 112907) was obtained from the National Cancer Institute, Bethesda, MD. FIAC, FIAU, FAC, FAU, FMAC, FMAU and [2-^{14}C]FIAC were synthesized by Fox and associates as previously described [1, 5]. IdCyd, IdUrd, dAdo, dGuo, dThd, dCyd, deoxyribonuclease I from bovine pancreas (type I), ribonuclease A (type III-A from bovine pancreas), phosphodiesterase I from *Crotalus atrox* venom (type VII), and *Escherichia coli* alkaline phosphatase (type III-R) were obtained from the Sigma Chemical Co., St. Louis, MO. [Methyl-^{14}C]thymidine and [2-^{14}C]dUrd were purchased from the New England Nuclear Corp., Boston, MA. Liquiscint was obtained from National Diagnostics, Inc., Somerville, NJ.

Animals

C57BL × DMA/2 F_1 (henceforth called $BD2F_1$) female mice were obtained from the A. R. Schmidt Co., Madison, WI, and were approximately 2 months old at the time of use.

Preparation of tissues

Small intestine. Each group of mice was injected i.p. with saline or with THU, 500 mg/kg. After 15 min, each mouse was injected i.p. with 1.8 mg (58.7 μCi) of [2-^{14}C]FIAC. They were killed at 24 hr, and the small intestines were removed, cleaned of intes-

tinal contents, and stored frozen until use. In a separate experiment, each mouse was injected i.p. with 0.1 mg (23 μCi) of [5-methyl-^{14}C]dThd or with 44.5 μg (6.1 μCi) of [2-^{14}C]IdUrd, 15 min after injection with saline, and thereafter treated in the manner described above.

P815 cells. P815 cells were maintained by passage in BD2F$_1$ mice. Approximately 2 × 10^6 cells were inoculated i.p. every 6 days for each passage. Mice were injected i.p. with saline or with THU, 500 mg/kg, at day 5 after inoculation. After 15 min, each mouse was given 0.27 mg (8.8 μCi) of [2-^{14}C]FIAC, i.p. The mice were killed 3 hr later. The ascites fluid was collected, and the cells were recovered by centrifugation at 2000 rpm and 4° for 10 min. The cells were stored frozen until use.

Purification of DNA

The extraction and purification of DNA from small intestine and P815 cells were carried out, using a modified phenol procedure [6]. The small intestine (or P815 cells) from each mouse was homogenized in a mixture of 5 ml of 88% aqueous phenol and 5 ml of 8% ammonium acetate. The homogenate was centrifuged, and the upper aqueous layer was removed and dialyzed to remove residual phenol. This fraction was mixed slowly with cold 95% ethanol, and the DNA was carefully spooled onto a narrow glass rod. The spooled DNA was dissolved in 0.01 M NaCl, and any contaminating RNA was removed by hydrolysis in 0.3 M KOH at 37° for 18 hr and then dialyzed. The DNA samples were neutralized, placed in a boiling water bath for 15 min to be denatured, and were rapidly cooled to prevent renaturation. Each sample was further purified by CsCl-gradient centrifugation in a Spinco L2 ultracentrifuge at 35,000 rpm, 20° for 48 hr, using an SW 50.1 rotor. Fractions were collected by bottom puncture of the tubes, and densities were determined from the refractive index which was measured with a Bauch & Lomb Abbe-3L refractometer. Aliquots were removed from each fraction and counted. A representative experiment showing the correspondence between absorption and radioactivity is shown in Fig. 1. The fractions containing the denatured DNA (equilibrium density of peak equal to 1.72 g/ml) were pooled, dialyzed, and frozen until needed.

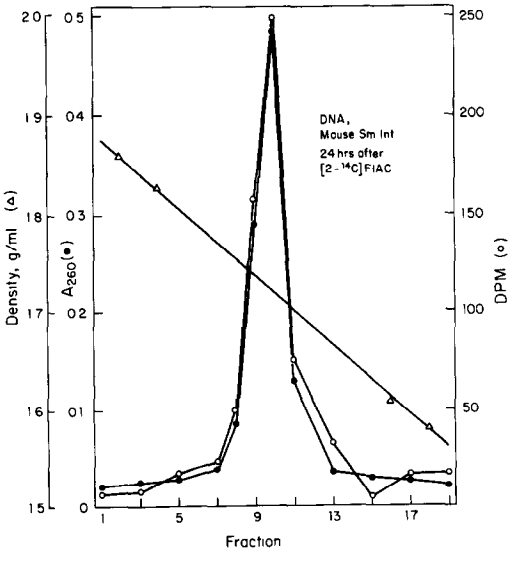

Fig. 1. Results of CsCl-gradient centrifugation of the DNA extracted from small intestine of mice treated with [2-^{14}C]FIAC (72 mg/kg, 32.6 μCi/mg, i.p., and killed 24 hr later). Fractions 9–11 were pooled, dialyzed to remove CsCl, and used for further treatment as described in the text. DNA from all other mice was purified in like manner using a similar gradient.

In several experiments, contaminating RNA was removed from intestinal DNA by digestion with ribonuclease A instead of KOH (see above). For these experiments, the pooled DNA was dissolved in 5 ml of 0.01 M NaCl and diluted with 4 ml of water and 1 ml of a solution of 0.15 M NaCl, 0.015 M sodium citrate, pH 7.3. Ribonuclease A (160 units) was added and the sample was incubated for 60 min at 37°. The DNA was precipitated by adding 1 vol. of 2 M NaCl and 2 vol. of ice-cold 95% ethanol and storing at −15° for 4 hr. The DNA was collected by centrifugation at 15,000 rpm, 4°, for 10 min and further purified by CsCl-gradient centrifugation as described above.

Enzyme digestions

The purified denatured DNA from each sample was digested with enzymes in the following manner. The volume of each solution was brought to 5 ml, adjusted to pH 7.5, and sufficient magnesium acetate was added to give a concentration of 15 mM. Three hundred units of DNAse I were added, and the solutions were incubated at 37° for 4 hr. The pH was then adjusted to 8.5; 0.05 units of phosphodiesterase I and 500 μg (per ml of solution) of bacterial alkaline phosphatase were added, and the samples were incubated at 37° for 18 hr. The solutions were deproteinized by adding 2 ml chloroform, shaking, and centrifuging at 10,000 *g* at 4° for 10 min. The upper aqueous layers were collected and stored frozen until chromatographic analysis.

High performance liquid chromatography

HPLC was performed using a Spectra-Physics (Piscataway, NJ) model 8000 liquid chromatograph, equipped with a data system, and a model 8300 fixed

Table 1. Mobile phase used in reversed phase HPLC*

Time (min)	% Methanol	% Phosphate buffer (0.02 M, pH 3.0)
0.0	2.0	98.0
10.0	2.0	98.0
11.0	7.0	93.0
14.0	7.0	93.0
15.0	35.0	65.0
17.0	35.0	65.0
18.0	10.0	90.0
40.0	10.0	90.0

* This table represents the solvent gradient used in all HPLC experiments involving the Partisil-10, ODS-3 column. The methanol and the water, used to prepare the buffer, were HPLC grade; both were obtained from the J. T. Baker Chemical Co., Phillipsburg, NJ.

Table 2. Incorporation of labeled dThd, IdUrd and FIAC into DNA of mouse small intestine and P815 ascites cells*

Tissue	Substrate	Dose (mg/kg)	± THU†	Radioactivity in DNA‡ (10^3 dpm/mg)	Incorporation of moles of radiolabel/10^4 moles of DNA nucleosides
Small intestine	[Me-^{14}C]dThd	4.0	−	222.1 ± 5.4	5.1 ± 0.15
Small intestine	[2-^{14}C]IdUrd	1.8	−	56.3 ± 0.3	1.7 ± 0.01
Small intestine	[2-^{14}C]FIAC	72.0	−	11.3 ± 1.7	2.2 ± 0.23
Small intestine	[2-^{14}C]FIAC	72.0	+	18.7 ± 1.7	3.6 ± 0.32
P815 cells	[2-^{14}C]FIAC	10.0	−	5.5 ± 0.1	1.1 ± 0.02
P815 cells	[2-^{14}C]FIAC	10.0	+	3.8 ± 0.1	0.7 ± 0.02

* Milligrams of DNA were determined by the diphenylamine reaction [7].

† Indicates whether or not THU (500 mg/kg) was injected i.p. 15 min before labeled substrate.

‡ The total amount of radioactivity (dpm) per mg of the DNA extracted from the tissue after purification on a CsCl gradient. Each number in the mean ± the average deviation for three mice when [2-^{14}C]FIAC (32.6 μCi/mg) was used and two mice when either [Me-^{14}C]dThd (230 μCi/mg) or [2-^{14}C]IdUrd (137 μCi/mg) was used. All compounds were injected i.p. Intestinal and P815 DNA were obtained, respectively, at 24 and 3 hr after injection.

wavelength detector. A Whatman (Clifton, NJ) Partisil 10, ODS-3, 4.6 × 250 mm prepacked column connnected to a Whatman guard column was used for all reverse phase analyses, with a flow rate of 1 ml/min and a column temperature of 40°. Absorbance of the eluted material was monitored at 254 nm. Radioactivity was determined by manually collecting 0.5 ml fractions into scintillation vials and adding 5 ml of Liquiscint. The mobile phase used consisted of a mixture of various amounts of methanol and 0.02 M potassium phosphate buffer, pH 3.0 (Table 1).

A Whatman Partisil 10, SCX prepacked column was used for cation exchange experiments. Samples were eluted isocratically at 1 ml/min with 0.02 M potassium phosphate buffer, pH 3.0, at 40°. As above, 0.5 ml fractions were collected and measured for radioactivity.

RESULTS

Incorporation of radiolabel into DNA

The incorporation of radioactivity from [2-^{14}C]FIAC into the DNA of mouse small intestine and P815 cells, with and without prior administration of THU, and the incorporation of labeled dThd and IdUrd are summarized in Table 2. The data show that for small intestinal DNA the incorporation of radioactivity was increased by THU treatment. For P815 cells, no increase in radiolabel incorporation was observed when THU was used. Table 2 also gives values for the moles of radiolabeled residues incorporated per 10^4 moles of DNA nucleosides. Although these values for intestinal DNA were of the same order of magnitude for labeled FIAC, dThd and IdUrd, the dose of FIAC given the mice was 18- and 40-fold higher than the dose of dThd and IdUrd respectively.

HPLC

To identify the radiolabeled nucleosides present, the enzyme-degraded samples of DNA from small intestine were resolved by HPLC. Figure 2 shows the HPLC chromatogram of enzyme-degraded DNA from the small intestine of a mouse given only [2-^{14}C]FIAC. Figure 3 shows the HPLC chromatogram of the nucleosides from small intestinal DNA of a mouse given both THU and [2-^{14}C]FIAC. The chromatograms indicate that the same three radioactive compounds were present in both samples; the compounds, in corresponding order of elution, were FAC, FMAU and FIAU. The percentages of each component for these two experiments are summarized in Table 3. It will be noted that the ratio of FAC to FIAU or to FMAU was higher after THU pretreatment than without THU. It should be emphasized that FIAC was not detected among the radiolabeled nucleosides.

The preparation of [2-^{14}C]FIAC used in these experiments contained a small amount of radioactivity (0.84%) with a retention time corresponding

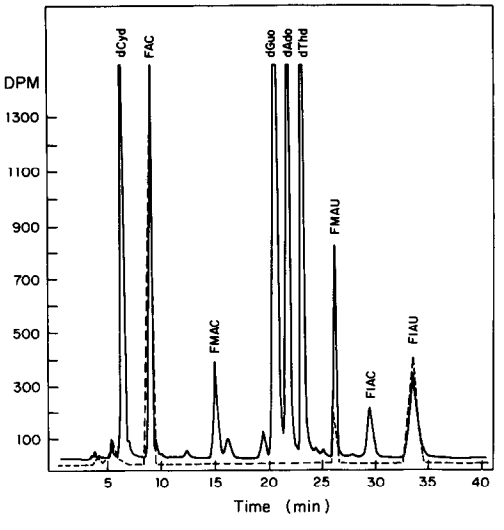

Fig. 2. Reverse phase HPLC chromatogram of enzyme-degraded DNA, from mouse small intestine after treatment with [2-^{14}C]FIAC (72 mg/kg, 32.6 μCi/mg, i.p. and killed 24 hr later) using a Partisil 10, ODS-3 column and a flow rate of 1 ml/min at 40°. The mobile phase consisted of methanol and 0.02 M potassium phosphate buffer (see Table 1) at varying relative concentrations (by volume) over the course of elution. The absorbance peaks of the non-labeled markers added to the radioactive sample as well as of the natural deoxyribonucleosides are identified. Key: solid line (———), absorbance at 254 nm; and dashed line (– – –), radioactivity (dpm).

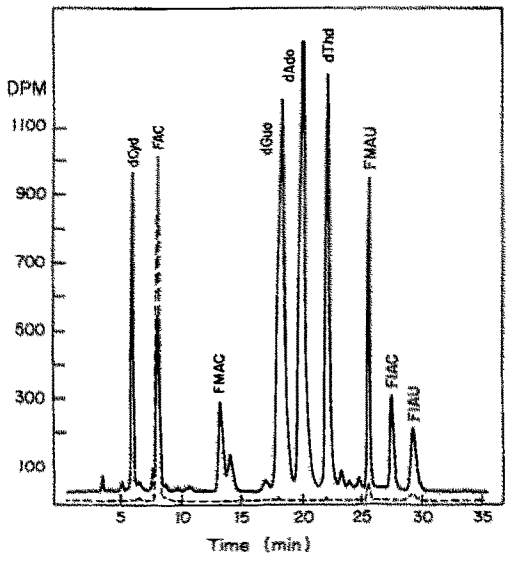

Fig. 3. Reverse phase HPLC chromatogram of enzyme-degraded DNA from mouse small intestine after treatment with 0.5 mg/kg THU (in saline) followed 15 min later by [2-^{14}C]FIAC (72 mg/kg, 32.6 μCi/mg, i.p., and killed 24 hr later). Chromatography was performed in the same manner as described in the legend of Fig. 2. Key: solid line (———), absorbance at 254 nm; and dashed line (– – –), radioactivity (dpm).

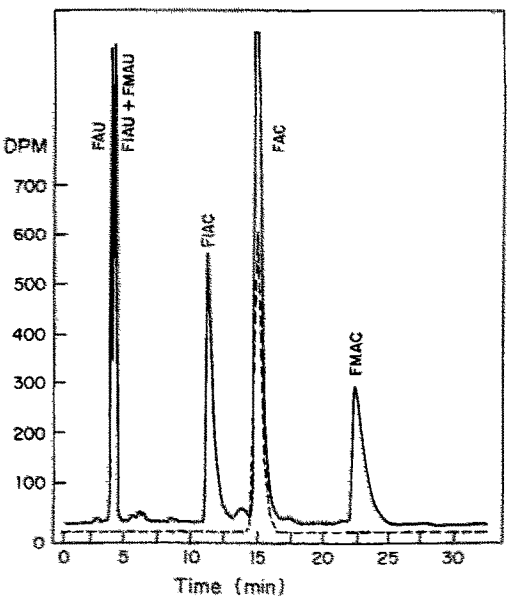

Fig. 4. Cation exchange HPLC chromatogram showing the retention time of the radiolabeled FAC collected by the reverse phase procedure of Fig. 2. The radioactive peak with retention time 9.0 min in Fig. 2 was collected and re-injected onto a Partisil 10, SCX column using 0.02 M potassium phosphate buffer, pH 3.0, as the mobile phase and a flow rate of 1 ml/min. Key: solid line (———), absorbance at 254 nm; and dashed line (– – –), radioactivity (dpm).

to FAC. Since FAC was found in the enzyme digest of DNA extracted from mouse small intestine, it was necessary to eliminate this impurity as a source of FAC for DNA synthesis. [2-^{14}C]FIAC was purified by using an already described HPLC procedure [5]. This purified material, with negligible quantities of FAC present [4], was injected into a mouse, and the DNA was extracted and purified as described above. HPLC analysis showed that the relative quantities of FAC, FMAU and FIAU in the enzyme-degraded DNA samples were the same as those of Table 3. Moreover, incorporation of radiolabel into the DNA was undiminished.

Table 3. HPLC analysis of enzyme-degraded DNA from mouse small intestine*

Sample	% Total radioactivity†	Corresponding marker
[2-^{14}C]FIAC (−THU)	7	Unknown
	60	FAC
	8	FMAU
	25	FIAU
[2-^{14}FIAC (+THU)	91	FAC
	5	FMAU
	4	FIAU
[2-^{14}C]IdUrd	3	Unknown
	37	dThd
	60	IdUrd

* Mice treated with [2-^{14}C]FIAC were given either saline (−THU) or 0.5 g/kg THU in saline (+THU), i.p., 15 min before the labeled substrate. [2-^{14}C]FIAC was given, i.p., 72 mg/kg, 32.6 μCi/mg. Mice treated with [2-^{14}C]IdUrd were given 1.8 mg/kg, 0.14 mCi/mg, i.p., in saline. No THU was used in the [2-^{14}C]IdUrd experiment. Intestine was obtained at 24 hr after injection.

† Total radioactivity used in each chromatogram was: FIAC (−THU), 3100 dpm; FIAC (+THU), 1217 dpm; and IdUrd, 7630 dpm.

The fact that FAC appeared to be present in the DNA suggested that FIAC can be deiodinated, either at the nucleoside or nucleotide level, without prior deamination. Since this was an unexpected result, additional chromatographic experiments were performed to further identify the substance and to exclude possible experimental artifacts.

The FAC fraction from an enzyme-degraded DNA sample from mouse small intestine was isolated by HPLC with the reverse-phase ODS-3 column. Elution was carried out using a water–methanol gradient to avoid accumulated salts. Fractions of 0.25 ml were collected and monitored for radioactivity. The fractions corresponding to FAC were combined and evaporated to dryness. The material was then taken up in 20 μl water and injected together with non-labeled markers onto the cation exchange column. Fractions (0.5 ml) were collected and counted. The data shown in Fig. 4 clearly demonstrate again that the radioactive compound was chromatographically identical with FAC. Preliminary study [3] using paper chromatography (isopropanol–HCl–water, 68:17:14.4, by vol.) showed that one of the hydrolytic products of small intestinal DNA has an R_f identical to FIAC. The present study using HPLC indicated that this component is, in fact, FAC which has an R_f value identical to that of FIAC in the paper chromatographic system.

A further analysis involved an attempt to hydrolyze the isolated [^{14}C]FAC fraction using strong acid

to liberate the pyrimidine base, [^{14}C]cytosine. HPLC analysis would have detected the latter in the acid hydrolysate. However, an authentic sample of FAC (and FIAC as well) was completely resistant to 6 N HCl at 100° for 60 min. This was probably due to the presence of the strongly electronegative fluoro group at the 2'-position which stabilizes the glycosyl linkage against acid hydrolysis. When the purified radioactive fraction was collected, exposed to the above hydrolysis conditions, and re-chromatographed by reverse phase HPLC, all of the radiolabel co-chromatographed with cold marker FAC. The acid stability of the radioactive moiety was further evidence for its identity with FAC.

The use of alkaline digestion to remove contaminating RNA during the purification of DNA was of some concern since incubation of FIAC with 0.3 M KOH at 37° for 18 hr was found to result in extensive changes in ultraviolet absorbance and since HPLC analysis of the KOH-treated FIAC revealed that the nucleoside had been largely converted to FAC and FIAU. To exclude the possibility that the FIAU and FAC present in DNA were artefacts due to KOH digestion, DNA from small intestine of mice given [2-^{14}C]FIAC was treated with ribonuclease A to remove RNA. HPLC analyses of the nucleosides prepared from ribonuclease-treated DNA provided results essentially similar to those reported in Figs 2 and 3 and Table 3.

Finally, in an effort to determine if some protein impurity in the enzyme preparations, used to hydrolyze the DNA samples, could have been responsible for deiodination of FIAC to FAC, FIAC was treated with each of the enzymes separately and with all three together under the conditions described (see Materials and Methods). Subsequent analysis by HPLC showed that only FIAC was present; no other products were detected.

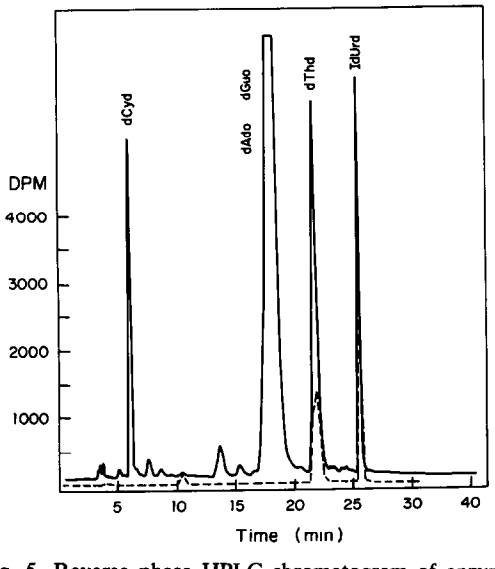

Fig. 5. Reverse phase HPLC chromatogram of enzyme-degraded DNA from mouse small intestine after treatment with [2-^{14}C]IdUrd (1.8 mg/kg, 0.14 mCi/mg, i.p., and killed 24 hr later). Chromatographic conditions were the same as in Fig. 2. Key: solid line (———), absorbance at 254 nm; and dashed line (– – –), radioactivity (dpm).

dThd and IdUrd

Digests of DNA from the small intestines of the mice of Table 2 given labeled dThd and IdUrd were also exmained by HPLC. Radioactivity was found only in the dThd peak in the animals that had received [Me-^{14}C]dThd. The HPLC analysis of the digests of DNA obtained after injection of [2-^{14}C]IdUrd is shown in Fig. 5 where the radioactivity appeared in peaks corresponding to both IdUrd and dThd. This result confirms the previous studies of Commerford and Joel [7], using Sephadex G10 and carboxycellulose chromatography, which showed that IdUrd can be deiodinated and methylated and, therefore, incorporated into DNA as thymidylate.

P815 DNA

Since the label of [2-^{14}C]FIAC was incorporated into intestinal DNA mostly as FAC (Table 3), it was of interest to determine if the same was true for the DNA of mouse P815 leukemic cells. HPLC analysis of the nucleosides resulting from enzyme degradation of the DNA of P815 cells from mice given [2-^{14}C]FIAC showed the same three metabolites that were present in mouse small intestinal DNA, namely, FAC, FMAU and FIAU. However, the precentages differed somewhat from those of intestinal DNA given in Table 3. In the DNA of P815 cells, FAC made up only 42.8% of the total radioactivity while FMAU and FIAU equalled 16.9 and 31.4% respectively. A total of 8.9% of the radioactivity was present in as yet unidentified peaks. A total of approximately 1000 dpm of radioactivity had been applied to the column in this experiment.

DISCUSSION

The present work demonstrates that the antiviral nucleoside, FIAC, is incorporated into mammalian DNA with the linkage between pyrimidine base and 2'-fluoro-1-β-D-arabinsofuranosyl moiety intact. However, the base is variously altered such that, rather than FIAC, three different nucleosides are present in DNA, namely, FIAU, FMAU, and FAC. These nucleosides have also been shown to be metabolites of FIAC in mice [3, 5]. Each of the three substances is, like FIAC, highly active against herpes virus [1].

The FIAU incorporated into DNA after giving mice FIAC probably arises in large part through deamination of FIAC by pyrimidine nucleoside deaminase and subsequent conversion of the deaminated product by kinases and phosphokinases into the triphosphate of FIAU. This is consistent with the results of Table 3 which show that the incorporation of FIAU was reduced in DNA of small intestine by pretreatment of mice with the pyrimidine nucleoside deaminase inhibitor, THU. We have also reported that FIAC is rapidly deaminated in mice and that the deamination can be nearly totally blocked by THU [3, 5]. If the monophosphate of FIAC is a substrate of deoxycytidylate deaminase, some conversion to FIAU monophosphate may also occur through the action of this enzyme. It is worth noting that previous workers have reported that structural analogs of FIAC such as 5-bromodeoxycytidine [6] and 5-iododeoxycytidine [8] are similarly

deaminated before serving as precursors of DNA synthesis.

The origin of FMAU is in all likelihood the result of deiodination and methylation of FIAU monophosphate through the action of thymidylate synthetase. There is an obvious analogy with one of the metabolic fates of IdUrd which has been shown recently to be incorporated into the DNA of mouse intestine as thymidylate [7], a result which is confirmed in the experiment of Fig. 5. The mechanism involved is understandable in the light of the data reported by Garrett *et al.* [9] which demonstrate that thymidylate synthetase from a *Lactobacillus* converts IdUrdMP to dUrdMP and methylates the latter, in the presence of methylene tetrahydrofolate, into TMP. We are currently studying whether the same enzyme can convert the monophosphate of FIAU into the nucleotide of FMAU. Preliminary results of this laboratory (unpublished observations) have confirmed the case.

The incorporation of FAC into DNA is understandable, for FAC is a close structural analog of the natural DNA precursor, dCyd. However, that FAC can be formed *in vivo* by deiodination of FIAC without prior deamination was an unexpected finding. Because of this, extra effort was directed toward proving the identity of the radiolabeled FAC in DNA of mice that had been treated with [2-^{14}C]FIAC. Thus, we accumulated evidence that (1) the peaks of radioactivity corresponded with that of marker FAC in both reversed phase and cation exchange HPLC systems, (2) known FAC and radioactive FAC fractions isolated by HPLC were both highly resistant to acid hydrolysis of the *N*-glycosyl bond, and (3) only negative results were obtained in control tests done to rule out the artefactural origin of FAC.

Our observation that FAC is one of the products of the reaction between FIAC and strong base (see above) indicates that it is chemically possible to deiodinate FIAC without removal of the 4-amino substituent. We presume that the reaction involves attack by hydroxide ion at carbon 6 with formation of the 5,6-iodohydrin. Nucleophilic attack by hydroxide ion on the 5-iodo group would follow, resulting in the formation of hypoiodite (IO^-) with generation of the 5,6 double bond and hydroxide ion. The nature of the enzyme system with capacity to catalyze the same reaction remains to be determined. We are presently studying the possibility that deiodination may be mediated through action of thymidylate synthetase on the monophosphate of FIAC in a manner similar to the deiodination of IdUrd monophosphate [9].

The results of Figs. 2 and 3 show that there is no detectable transfer of radioactivity into the natural deoxyribonucleosides of DNA in mice given [2-^{14}C]FIAC. This is in keeping with our previous findings that the total degradation is only a minor metabolic fate of FIAC in mice [5]. Clearly, ^{14}C-containing fragments of the pyrimidine ring are not appreciably utilized for synthesis of nucleic acid bases.

Acknowledgements—This work was supported, in part, by National Cancer Institute Grants CA 18856, CA 27569, CA 18601 and CA 08748 and by the Elsa U. Pardee Foundation. A. G. is a postdoctoral Fellow supported by CA 09207 from the National Cancer Institute.

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