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BIOCHEMICAL AND PHARMACOLOGICAL STUDIES WITH 1-β-D-ARABINOFURANOSYLCYTOSINE IN MAN*

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Abstract—Cytosine arabinoside, labeled with tritium, was administered to five patients with far-advanced neoplasms. The analog was rapidly deaminated to uracil arabinoside; the latter accounted for between 86 and 96 per cent of the radioactivity excreted in the urine. Incorporation of tritium-labeled cytidine into DNA by suspensions of human leukemic leukocytes was inhibited in the presence of cytosine arabinoside. Although administration of cytosine arabinoside at therapeutic levels depressed the ability of leukemic leukocytes to incorporate cytidine into DNA *in vitro*, there was no correlation between the degree and duration of this effect and the clinical response to the drug. Tritium-labeled cytosine arabinoside entered human leukemic leukocytes very rapidly when incubated with the cells *in vitro*; there was a small but significant incorporation of the analog into both DNA and RNA.

1-β-D-ARABINOFURANOSYLCYTOSINE (cytosine arabinoside; ara-C) exerts antitumor acitivity against both rodent[1] and human[2] neoplasms. The studies of Chu and Fischer[3] implicated an inhibition of the conversion of cytidine diphosphate to deoxycytidine diphosphate as the primary site of action of ara-C in murine lymphoblast cells L5178Y; the inhibitory effect of the analog upon viral and tumor systems could be prevented both *in vitro*[3, 4] and *in vivo*[5] by deoxycytidine. More recently it was suggested that there may be other biochemical lesions resulting from the incorporation of ara-C into nucleic acids.[6]

Preliminary studies in patients with neoplastic disease have confirmed the antineoplastic activity and the production of megaloblastic changes in the bone marrow;[7] the development of the latter changes was largely prevented by early administration of deoxycytidine. In addition, it was reported briefly that ara-C was rapidly degraded to uracil arabinoside (ara-U), presumably by a pyrimidine nucleoside deaminase[8] that occurs in a number of human tissues,[9] without, however, undergoing further cleavage. This paper describes metabolic studies with ara-C and shows that in human leukemic cells the drug inhibits the incorporation of pyrimidine ribonucleosides into DNA, and is itself incorporated into nucleic acids.

MATERIALS AND METHODS

Chemicals. 1-β-D-Arabinofuranosyl-cytosine and -uracil were supplied by Doctors James H. Hunter and Charles G. Smith of the Upjohn Co. Tritium-labeled ara-C

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was obtained from Schwartz BioResearch, Inc., and, before use, was purified by paper chromatography in the *n*-butanol:formic acid and *n*-butanol:ammonia systems described below. ^{3}H-Cytidine and ^{3}H-uridine were purchased from the New England Nuclear Corp. All three nucleosides had 60–75 per cent of the tritium at the 5-position of the pyrimidine ring and essentially none in the pentose.

Metabolic studies. Ara-C containing 7·3 to 27·7 $\times$ 10^6 counts/min of tritiated drug was administered intravenously at a dosage of either 5 or 10 mg/kg to five patients for whom the relevant clinical information appears in Table 1. Blood samples

TABLE 1. SUMMARY OF CLINICAL DATA REGARDING PATIENTS WHO RECEIVED ^{3}H-CYTOSINE ARABINOSIDE*

Number	Age	Sex	Diagnosis	Dose (mg/kg)	Route
1	47	M	Malignant melanoma	5	i.v. injection
2	36	F	Malignant melanoma	10	i.v. injection
3	61	M	Malignant melanoma	10	i.v. injection
4	46	M	Reticulum cell sarcoma	5	i.v. infusion for 10 hr
5	22	M	Hepatoma	5	i.v. infusion for 10 hr

* Cytosine arabinoside containing between 7·3 and 27·7 $\times$ 10^6 counts/min of tritium-labeled compound was administered by the specified route.
Abbreviations: M, male; F, female; i.v., intravenous.

(5 ml) were removed by venipuncture, and a portion was acidified with an equal volume of cold perchloric acid (1·0 M). After centrifuging for 5 min at 1600 *g*, aliquots of the supernatant material were assayed for radioactivity in a Packard Tri-Carb scintillometer; the remainder was neutralized with potassium hydroxide to precipitate the perchlorate ion, and subjected to paper chromatography as described below. A portion of the fresh blood was centrifuged to separate the plasma, which was then acidified with an equal volume of perchloric acid (1·0 M), and the supernatant assayed for radioactivity. Urine was collected at intervals during a 48-hr period, and the metabolites were studied by paper chromatography.

Tissues. Preparations of human leukemic leukocytes were obtained by withdrawing between 20 and 50 ml blood, adding heparin, as well as a solution of dextran to give a final concentration of 3%, and allowing the erythrocytes to sediment for 1 hr at 4°. The suspension above the erythrocyte layer was carefully withdrawn and centrifuged initially at 300 *g* for 5 min to separate the platelets that remained in the supernatant; after resuspension in saline, the leukocytes were sedimented at 1600 *g* for 5 min. Residual erythrocytes in the cell pellet were lysed by exposure to cold distilled water for 20 sec before restoration of isotonicity by addition of a 3·5% solution of sodium chloride. Leukocytes were finally washed and resuspended in Eagle's medium supplemented with 10 per cent of calf serum (Microbiological Associates) and glutamine (2 μmoles/ml). Suspensions of bone marrow cells were obtained in a similar manner from aspirates drawn from the iliac crests. For comparative studies, mouse lymphoblast L5178Y cells were employed; these cells were carried in the ascitic form in C57BL $\times$ DBAF1 mice. Contaminating erythrocytes were lysed by brief exposure to hypotonic saline (0·2%), and the cells were finally washed in the incubation medium described above.

Incorporation studies. Cells (0·03–0·05 ml packed cells) were incubated for 30 min in the supplemented Eagle's medium, with or without ara-C or deoxycytidine. At the end of this preincubation, 1 μc ^{3}H-uridine (3·6 mc/μmole) or ^{3}H-cytidine (3·5 mc/μmole), or 4 μc ^{3}H-cytosine arabinoside (2·5 mc/μmole) was added to each flask, making a final volume of 2 ml, and incubation was continued for various times up to 1 hr. At the end of the reaction time, the incubation mixture was carefully layered over 4 ml of sucrose solution (0·25 M) in Shevky-Stafford and McNaught centrifuge tubes, and centrifuged for 3 min to separate the cells from the radioactive medium. Incubation medium and sucrose solution were removed by aspiration, and the cell pack was suspended in a small amount of cold perchloric acid (0·5 M) and collected with a Pasteur pipette. This cell pack was washed four times with cold perchloric acid to remove all the cold acid-soluble intracellular radioactivity, and the residue resuspended in 1 ml of NaOH solution (0·2 N). The suspension was heated to 90° for 30 min to hydrolyze RNA, cooled to 0°, and acidified with perchloric acid to a final concentration of 0·5 M. After centrifuging to remove DNA and protein, and re-extracting the residue with perchloric acid, the supernatants containing the ribonucleotides derived from RNA were combined and assayed for radioactivity (Packard Tri-Carb scintillometer) and absorbance at 260 mμ. DNA remaining in the residue was hydrolyzed by heating for 30 min with 0·5 M perchloric acid at 80°, and the radioactivity and absorbance at 260 mμ released into the supernatant fraction were determined. Measurements of absorbance at 260 mμ were closely correlated with the amounts of RNA, as determined by the orcinol method,[10] and of DNA, measured by the diphenylamine procedure;[11] determination of RNA and DNA in small tissue samples by measuring ultraviolet absorbance has been described previously.[12] In certain experiments, in which the scale of the incubations was increased fivefold, RNA was not hydrolyzed with alkali but was separated into subfractions by extraction with phenol and precipitation with lithium chloride, as described previously.[13]

Chromatography. Paper chromatography was carried out in descending fashion on Whatman no. 1 paper in *n*-butanol:formic acid:water (77:10:13, v/v) or *n*-butanol:water:23 N ammonium hydroxide solution (81·5:13:5·5, v/v) as the solvents. Ara-C and its phosphate esters were separated on columns (2·5 ml) of ecteola previously washed with alkali, acid and water, and adjusted to pH 2·7.[6] Acid-soluble extracts were neutralized immediately with 6 N potassium hydroxide, centrifuged at 0° to remove potassium perchlorate, and aliquots (0·5 or 1·0 ml) applied to the columns; unlabeled samples (0·2 μmole) of ara-C, dCMP, and dCTP were added as carriers. Elution was carried out with a sequence of water followed by 0·01, 0·02, and 0·3 M hydrochloric acid. The identity of the phosphate esters of ara-C, in view of the small amounts involved, was not established by any other means than their chromatographic behavior.

RESULTS

Metabolic studies

After single intravenous injections of ara-C, the levels of radioactivity in the blood, determined in the cold acid-soluble form, fell rapidly, with a half-life of 30–60 min (Fig. 1). For the patients who received ara-C by an intravenous infusion over a 10-hr period, the amounts of tritium in the blood stabilized at levels less than 10 per cent of the maximal concentrations achieved after rapid single injections. Levels

of radioactivity in the plasma were routinely higher than in whole blood. Examination of the acid extracts of whole blood by paper chromatography failed to reveal ara-C itself more than 5 min after injection in two subjects, and after 20 min in a third. The only metabolite found had the chromatographic behavior of uracil arabinoside (ara-U), and when eluted from the paper the substance had an ultraviolet absorption

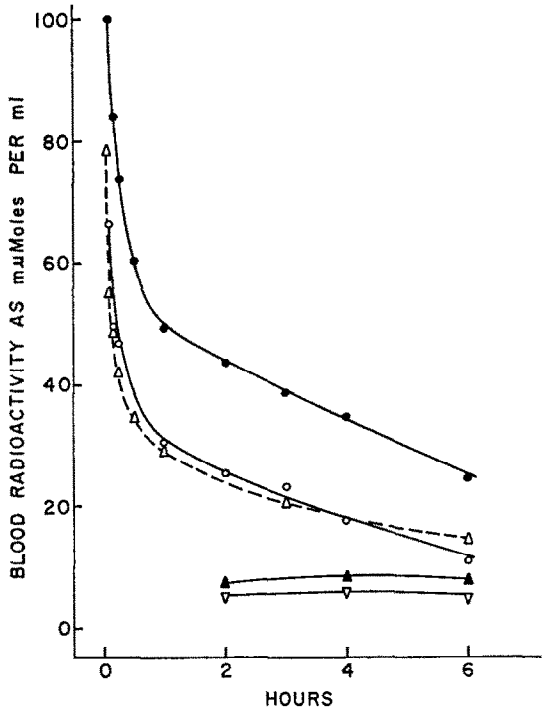

FIG. 1. The rate of fall in the levels of radioactivity in whole blood after administration of ^{3}H-cytosine arabinoside. Results are expressed in terms of mμmoles of drug in view of the variation in administered radioactivity and of the fact that only nucleosides occur in the acid extracts of whole blood. Patient numbers are: △- - -△, 1; ○———○, 2; ●———●, 3; ▽———▽, 4; ▲———▲, 5; Patients 4 and 5 received the drug by infusion over a 10-hr period.

spectrum identical with that of ara-U. Urinary excretion of radioactivity was rapid and essentially complete within a period of 24 hr (Fig. 2). Only two radioactive materials were seen in the urine when the administered compound had been rigorously purified; these exhibited the spectral and chromatographic characteristics of ara-C and ara-U. The kinetics of the urinary excretion of these two compounds appear in Fig. 3. A summary of the excretion data, including amount and form of the radioactivity, is presented in Table 2.

Cytidine and uridine incorporation

Entry of ^{3}H-cytidine into the acid-soluble fraction of all the cell types studied, including bone marrow, peripheral leukocytes, and L5178Y cells, reached a plateau within about 20 min, whereas incorporation of isotope into DNA proceeded linearly

for at least 60 min, and into RNA for about 40 min (Fig. 4). Rates of incorporation into acid-soluble and RNA fractions were comparable and approximately one hundred times greater than uptake into DNA. It is of interest that the entry of radioactivity from cytidine into DNA was between five and twenty times greater in mouse lymphoblasts than in several varieties of human leukemic leukocytes, whereas the uptake of

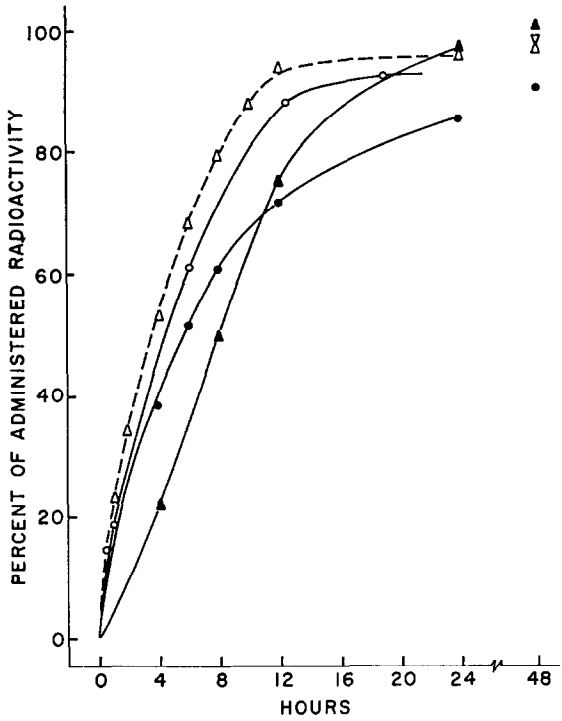

FIG. 2. The rate of urinary excretion of radioactivity during either a 19-hr or 48-hr period after the administration of ^{3}H-cytosine arabinoside. Patient identification is as follows: △———△, 1; ○———○, 2; ●———●, 2; ▽———▽, 4; ▲———▲ 5. Patients 4 and 5 received the drug by infusion during a period of 10 hr at a dose of 5 mg/kg; the other patients received the drug by injection at doses of 5 mg/kg (number 1) or 10 mg/kg (numbers 2 and 3).

uridine into the DNA of murine cells was at the same level as that of cytidine into the DNA of the same types of human cells or of uridine into the one type of human cells tested (Table 3). Preincubation of the cells with unlabeled ara-C markedly reduced the incorporation of ^{3}H-cytidine into DNA (Fig. 5); L5178Y cells appeared to be more sensitive than the human cells. A similar though less pronounced depression was noted in the uptake of ^{3}H-uridine into DNA; this inhibition reflected, presumably, a defficiency of deoxycytidine triphosphate needed for DNA synthesis. The entry of ^{3}H-uridine and ^{3}H-cytidine into the acid-soluble fraction and RNA was not inhibited by pretreatment with ara-C. An attempt was made to demonstrate this inhibitory action in two patients who received therapeutic levels of ara-C; incorporation of ^{3}H-cytidine into their leukocytes was assayed *in vitro* before and during administration of drug. In one subject (Patient 6) with a diagnosis of acute monocytic leukemia, the rate of incorporation of the nucleoside into DNA, which was initially 1·27 μμmoles/

0·1 ml cells/hr, was inhibited 97 per cent by ara-C (5 mg/kg/day × 3). Although this suppression persisted until 3 days after therapy ceased, there was no significant reduction in leukocyte count, and the patient expired through progression of his disease. In Patient 7, with acute myelomonocytic leukemia, a 49 per cent depression in the incorporation of ^{3}H-cytidine into DNA disappeared by the fourth day of therapy

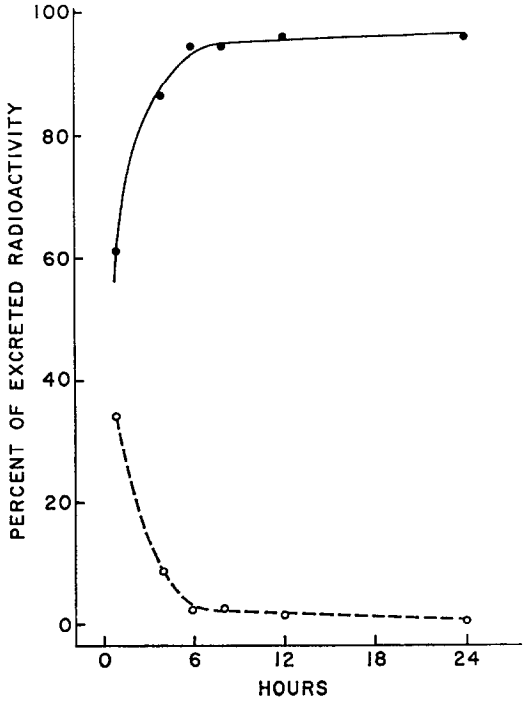

FIG. 3. The time course of the changes in distribution of urinary radioactivity after administration to Patient 3 of 10 mg cytosine arabinoside per kg; the drug contained 17·7 × 10^{6} counts/min of tritium-labeled compound. Metabolites were separated by paper chromatography as described in Methods; ●——●, uracil arabinoside; ○——○, cytosine arabinoside.

TABLE 2. URINARY EXCRETION PRODUCTS OF ^{3}H-CYTOSINE ARABINOSIDE

Patient No.	Route of administration*	Tritium excreted (% of administered)†	Per cent of urinary ara-C	Composition products ara-U‡
1	Injection	98·2	8·4	92·6
2	Injection	92·2	6·5	91·7
3	Injection	88·9	10·0	86·0
4	10-hr infusion	97·3	7·0	92·6
5	10-hr infusion	100·2	3·8	96·0

* ^{3}H-Cytosine arabinoside was administered either as a single injection or as an intravenous infusion over a 10-hr period.

† This represents the total urinary excretion during a 48-hr period of radioactivity; expressed as per cent of the administered dose.

Abbreviations: ara-C, cytosine arabinoside; ara-U, uracil arabinoside.

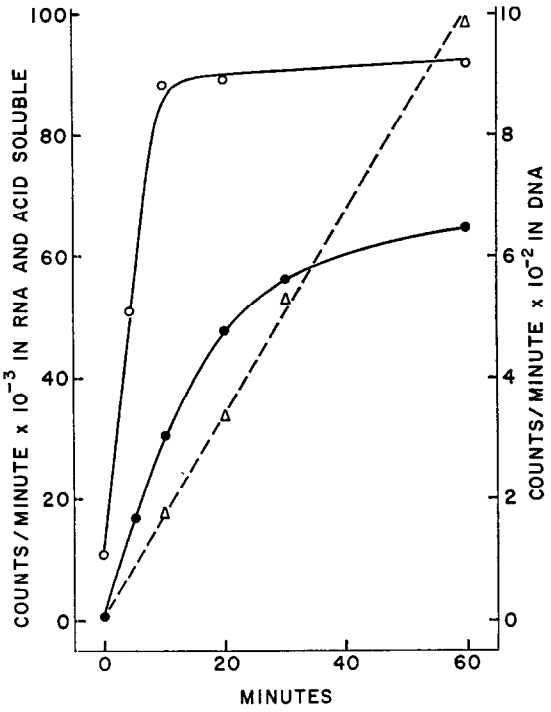

FIG. 4. The uptake of ^{3}H-cytidine by human leukemic leukocytes (chronic granulocytic leukemia) *in vitro*. Incubation conditions were as described under Methods, and the results are indicated as follows: ○———○, uptake into the acid-soluble fraction; ●———●, incorporation into RNA; △- - -△, incorporation into DNA. Data are expressed as counts/min incorporated into the specified fractions derived from 0·04 ml of packed cells.

TABLE 3. UPTAKE OF ^{3}H-CYTIDINE AND ^{3}H-URIDINE INTO THE NUCLEIC ACIDS OF HUMAN LEUKEMIC LEUKOCYTES AND MOUSE LYMPHOBLASTS*

Cell type	Incorporation (μμmole/0·1 ml cells/hr)			
	^{3}H-Cytidine		^{3}H-Uridine	
	DNA	RNA	DNA	RNA
Acute monocytic	1·27	49·8		
Acute monocytic (bone marrow)	1·24	93·7		
Chronic granulocytic	1·60	107·0		
Chronic granulocytic	1·23	64·9		
Acute myelomonocytic	0·83	160·4		
Acute myelomonocytic	1·18	180·7	1·03	310·2
Subacute lymphoblastic	0·68	53·4	0·26	88·7
L5178Y lymphoblasts	8·09	228·7	0·91	145·9

* Cells (approximately 0·05 ml packed cell volume) were incubated in Eagle's medium, supplemented with calf serum and glutamine, as described in Methods. ^{3}H-Cytidine or ^{3}H-uridine (3·6 or 3·5 mc/μmole) was present at a level of 1 μc [approximately $1·5 \times 10^5$ counts/min] per 2 ml. Packed cell volumes and DNA content were measured in each experiment, but the data were calculated on the basis of 0·1 ml packed cell volume; this enabled direct correlation of uptake into all fractions, including the acid soluble.

(1 mg ara-C/kg daily by continuous infusion) when the activity rose above the baseline level; simultaneously, there was a precipitous drop in the peripheral leukocyte level (Fig. 6). Deoxycytidine may prevent the action of ara-C, both by supplying deoxycytidine phosphates needed for the synthesis of DNA, and by competing with analog at the kinase sites.[6] The effect of deoxycytidine on the uptake of ^{3}H-cytidine into DNA

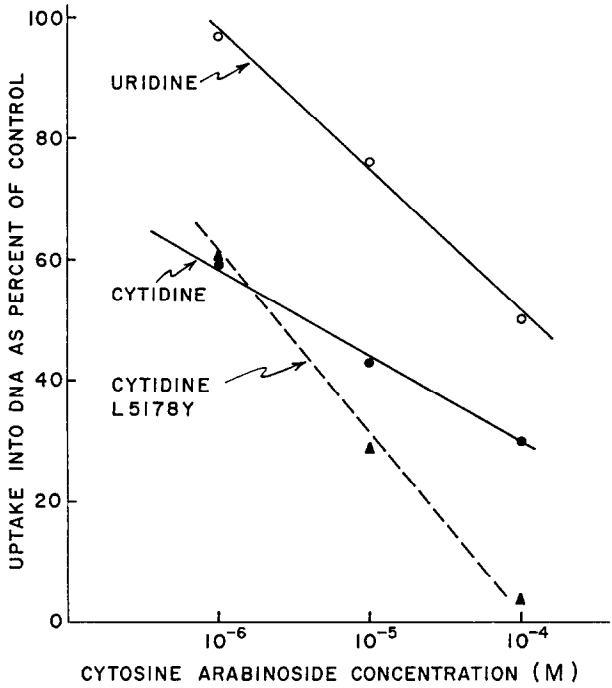

FIG. 5. The inhibition by cytosine arabinoside of the incorporation of cytidine and uridine into DNA. Values are expressed on the basis of percentage of control uptake and represent the means of two experiments with murine L5178Y cells and five experiments with human leukocytes. Human leukocytes: cytidine uptake ●———●, uridine uptake ○———○; L5178Y cells: cytidine uptake ▲———▲. Cells were preincubated with or without ara-C for 30 min before the addition of labeled precursor.

in the presence and absence of ara-C was studied in both human and mouse cells (Table 4). As would be expected, deoxycytidine reduced the incorporation of ^{3}H-cytidine by a process of dilution but, in addition, it appeared to antagonize the inhibitory effect of the arabinoside, so that the inhibition caused by the combination was usually less than that produced by ara-C alone. This effect was not seen over a complete range of concentrations because of the inhibitory action of both compounds in large excess.

Incorporation of cytosine arabinoside

The uptake of ^{3}H-ara-C into the acid-soluble fraction of both leukocytes and bone marrow cells proceeded rapidly and to an extent comparable to that seen with ^{3}H-cytidine. RNA took up the label from ara-C at a far lower rate than was the case with ^{3}H-cytidine, while with DNA, ^{3}H-cytidine was incorporated, under the same

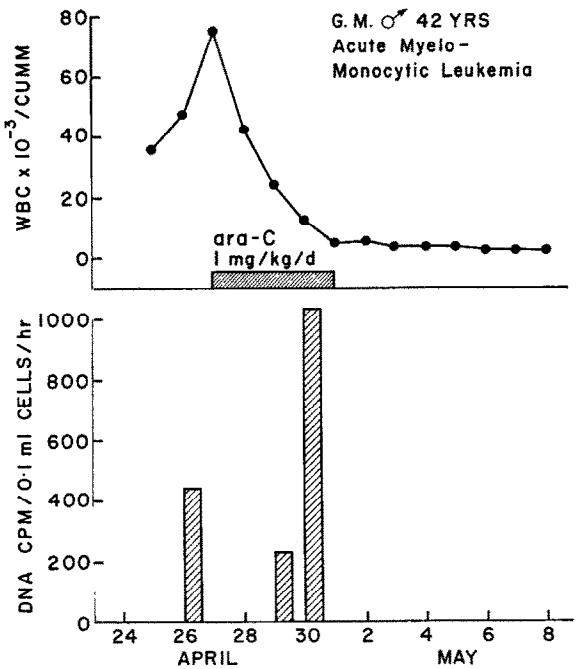

FIG. 6. Reduction in peripheral leukocyte count and changes in uptake of ^{3}H-cytidine into leukocyte DNA in a patient treated with cytosine arabinoside (ara-C).

TABLE 4. THE EFFECT OF DEOXYCYTIDINE ON THE INHIBITION BY CYTOSINE ARABINOSIDE OF THE UPTAKE OF CYTIDINE INTO DNA*

Cell type	Concentration (M)		Incorporation (counts/min/ 0·1 ml cells/hr)
	ara-C†	dCR†	
Chronic granulocytic leukocytes	0	0	659
	10^{-6}	0	329
	10^{-4}	0	100
	0	10^{-5}	379
	0	10^{-3}	180
	10^{-6}	10^{-5}	421
	10^{-6}	10^{-3}	243
	10^{-4}	10^{-5}	136
	10^{-4}	10^{-3}	176
Acute myelomonocytic leukocytes	0	0	444
	10^{-6}	0	227
	10^{-5}	0	151
	0	10^{-5}	286
	10^{-6}	10^{-5}	232
	10^{-5}	10^{-5}	227
L5178Y lymphoblasts	0	0	7580
	10^{-5}	0	934
	0	10^{-5}	7298
	0	10^{-3}	2560
	10^{-5}	10^{-5}	1898
	10^{-5}	10^{-3}	2818

* Cells (approximately 0·05 ml packed cell volume) were preincubated in Eagle's medium, supplemented as described in Methods, together with ara-C and dCR as indicated. ^{3}H-Cytidine was then added at a level of 1 μc (approximately $1·5 \times 10^{5}$ counts/min of 3·6 mc/μmole) per 2 ml. Preincubation and incubation times were 30 and 40 min respectively.

† Abbreviations: ara-C, cytosine arabinoside; dCR, deoxycytidine.

conditions, to an extent two to four times as great as with ara-C (Table 5). When the form of the radioactivity in the acid-soluble fraction was examined, it was found that in human leukemic cells, as in L5178Y cells,[6] the larger proportion of the radioactivity was present as phosphorylated derivatives of ara-C (Table 6). Examination of the RNA subfractions indicated that soluble RNA had a specific activity about 20 per cent

TABLE 5. THE INCORPORATION OF ^{3}H-CYTOSINE ARABINOSIDE INTO THE NUCLEIC ACIDS OF HUMAN LEUKEMIC LEUKOCYTES*

Cell type	Incorporation ($\mu\mu$mole/0·1 ml cells/hr)	
	DNA	RNA†
Chronic granulocytic	0·32	0·89
Chronic granulocytic	0·83	1·57
Acute myelomonocytic	1·02	4·48
Acute myelomonocytic	1·27	2·68

* Cells (approximately 0·05 ml packed cell volume) were incubated in Eagle's medium, supplemented with calf serum and glutamine, as described in Methods. ^{3}H-Cytosine arabinoside (2·5 mc/μmole) was present at a level of 4 μc (approximately 6×10^5 counts/min) per 3 ml.

† The extent of uptake into RNA should be compared with the figures for incorporation of ^{3}H-cytidine, which fall in the range 49·8 to 180·7 $\mu\mu$moles/0·1 ml cells/hr (Table 3). The tissues were derived from the same samples as those in Table 3.

TABLE 6. THE PHOSPHORYLATION OF ^{3}H-CYTOSINE ARABINOSIDE BY LEUKEMIC LEUKOCYTES*

Tissue	Time (min)	Level in acid-soluble ($m\mu$moles/0·1 ml cells)	Percentage composition†		
			ara-C	ara-CMP	ara-CDP ara-CTP
Chronic granulocytic leukemia	20	0·27	56·6	7·5	35·9
	60	0·42	39·1	30·9	30·0
Acute myelomonocytic leukemia	10	0·43	79·2	10·7	10·1
	20	0·92	15·1	24·0	60·9

* Incubations were carried out as described in Table 4 and Methods (0·033 ml cells).

† Abbreviations: ara-C, cytosine arabinosides; ara-CMP, ara-CDP, ara-CTP, mono-, di-, and triphosphates of cytosine arabinoside.

higher than that of ribosomal RNA; this would not be unexpected if ara-C were incorporated into the rapidly-turning over pCpCpA end groups.[14] Alkaline hydrolysis of RNA labeled with ^{3}H-ara-C, followed by chromatography of the product on ecteola columns and paper, failed to disclose significant amounts of free ara-C; i.e. the radioactivity migrated as a nucleotide. After treatment with phosphatases, radioactivity was released that behaved as ara-C; this accounted for about 20 per cent of the total tritium, most of the remainder having properties consistent with an oligonucleotide. A small amount of radioactivity, of the order of 30 per cent of that

associated with ara-C, migrated with ara-U, but, since significant deamination of ara-C occurred under the same conditions of alkaline hydrolysis, it cannot be said that this tritium represents ara-U in RNA.

DISCUSSION

The remarkable rapidity with which ara-C is deaminated in man represents the most striking finding in the present study; this was not entirely unexpected, however, in view of the previous demonstration of the rapid conversion of 5-iodo-2′-deoxycytidine to the corresponding derivative of uracil.[15] Since the halogenated nucleoside was administered at dosages eight to sixteen times larger than those of ara-C, the latter might be expected to persist for a proportionately shorter time in the blood stream (if the same or a similar deaminase is involved); in fact, the findings are in agreement with this hypothesis. A similarly high rate of deamination has been demonstrated in bacterial cultures,[16] in mice,[17] and in cultures of mammalian cells.[18] Nevertheless, despite its rapid conversion to ara-U, which is apparently not an effective inhibitor of cell growth,[3] ara-C does exhibit inhibitory activity against tumors and exerts pronounced suppressive effects on the bone marrow; this could be attributed to very rapid sequestration by cells. Certainly, both leukemic and normal cells in the bone marrow take up the analog very rapidly and convert it extensively to phosphorylated derivatives. Once present intracellularly as phosphate esters, the drug would not be subject to the action of nucleoside deaminases and, although deoxycytidylate deaminase might act upon it, this latter enzyme is present in relatively few tissues.[19]

The data presented here are particularly relevant to the fate of the phosphorylated derivatives of ara-C. A demonstration of the potential of such derivatives for inhibiting the enzymatic reduction of cytidine diphosphate to the corresponding derivative of deoxycytidine in human cells emerges from the results of the experiments on uptake of ^{3}H-cytidine into DNA. It is, however, especially interesting that this pathway appears to be approximately ten times less active in such cells than in L5178Y lymphoblasts. This might imply that human leukemic leukocytes and bone marrow cell populations are more dependent on externally supplied deoxyribonucleosides than are the murine lymphoblast cells. If this were so, and in view of the lesser sensitivity of the reductive step to ara-C in human cells, it might be asked whether inhibition of this conversion is the major site of action of the compound. Another agent, 6-uracil methyl sulfone, which interferes with such a reductive step, in this case on the pathway of uridine uptake into DNA,[20] unlike ara-C, is not a particularly effective inhibitor of the growth of tumors.[21] Furthermore, in Patient 7 we have seen that a marked and long-lasting depression of the peripheral leukocyte level was achieved under conditions in which the inhibition at the diphosphate level had been released. In this context, incorporation of the analog into nucleic acid seems to acquire added importance. Although it has been claimed that in isolated enzyme systems the triphosphate of ara-C is not a substrate for DNA and RNA polymerases,[22] incorporation of the nucleoside, without loss of the arabinose moiety, into mammalian cell nucleic acids has been clearly demonstrated,[6, 23] and the present data extend this finding to human cells. The greatest activity in terms of the phosphorylation and incorporation of ara-C was displayed by acute myelomonocytic leukocytes, a cell type previously shown to be very active in pyrimidine synthesis *de novo*.[24] Although the degree of incorporation was rather low, reaching approximately one molecule of ara-C per two million nucleotide residues

in DNA, and four times this value in RNA, at present it is not known how much incorporation is lethal for a cell in appropriate circumstances.

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