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Research Article

STUDIES ON NORMAL AND LEUKEMIC LEUKOCYTES. VI. THYMIDYLATE SYNTHETASE AND DEOXYCYTIDYLATE DEAMINASE *

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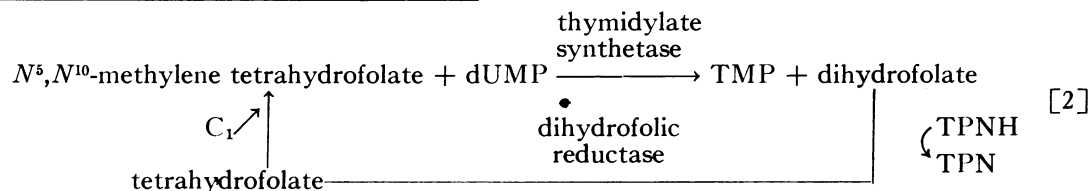
Thymidylate, a key precursor of DNA,[1] is synthesized by the tetrahydrofolate-dependent methylation of deoxyuridylate or by the phosphorylation of thymidine via thymidine kinase (3). The former reaction, catalyzed by thymidylate synthetase, has been the subject of several investigations (4–6) including that of Wahba and Friedkin (7, 8), who established the stoichiometry shown in Equation 1.

$$N^5,N^{10}\text{-methylene tetrahydrofolate} + \text{dUMP} \rightarrow \text{TMP} + \text{dihydrofolate} \qquad [1]$$

The C_1 unit is transferred from N^5,N^{10}-methylene tetrahydrofolate to dUMP, and this reductive methylation step is coupled with the oxidation of tetrahydrofolate to dihydrofolate. Dihydrofolate is then recycled to tetrahydrofolate by the action of dihydrofolic reductase, and the addition of a C_1 unit, possibly via serine hydroxymethylase, completes the "thymidylate cycle" as outlined in Equation 2.

$$N^5,N^{10}\text{-methylene tetrahydrofolate} + \text{dUMP} \xrightarrow[\text{synthetase}]{\text{thymidylate}} \text{TMP} + \text{dihydrofolate} \qquad [2]$$

(with C_1 pathway via tetrahydrofolate, and dihydrofolic reductase coupled to TPNH/TPN)

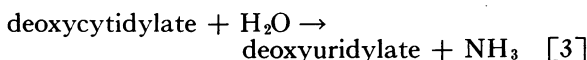The present investigation is concerned with the characterization of thymidylate synthetase from human leukocytes and with the levels of this enzyme in normal and leukemic leukocytes. Studies are also presented on an ancillary enzyme, deoxycytidylate deaminase, which is responsible for the conversion of dCMP to dUMP via Equation 3.

$$\text{deoxycytidylate} + H_2O \rightarrow \text{deoxyuridylate} + NH_3 \qquad [3]$$

METHODS

The following materials were used: C^{14}-formaldehyde (10 µc per µmole) ; [2] DNA; [3] protamine sulfate, uracil, uridine, UMP, dUMP, thymine, thymidine, TMP, TTP, cytosine, cytidine, deoxycytidine; 5-methyl deoxycytidine, CMP, CDP, CTP, dCMP, dCDP, dCTP, cytidine-2′, 3′-cyclic phosphate, and 6-thioguanine; [4] aminopterin; [5]

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[1] Abbreviations: UMP, uridine-5′-phosphate; dUMP, deoxyuridine-5′-phosphate; CMP, CDP, CTP, cytidine mono-, di-, and triphosphate; dCMP, dCDP, dCTP, deoxycytidine mono-, di-, and triphosphate; TMP, TTP, thymidine mono- and triphosphate; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; Tris, tris(hydroxymethyl)aminomethane; FUDR, fluorodeoxyuridine; FUDRP, fluorodeoxyuridine-5′-phosphate; DEAE, diethylaminoethyl; C_1, 1-carbon unit; AML and CML, acute and chronic myelocytic leukemia; ALL and CLL, acute and chronic lymphocytic leukemia.

Dowex 50W-X4(H⁺) and Dowex 1-X10(Cl⁻) ; [6] hydroxide of Hyamine 10X, POPOP [1,4-bis-2-(5-phenyloxazolyl)benzene] and PPO (2,5-diphenyloxazole) ; [7] 6-mercaptopurine; [8] FUDR and 3'-acetyl-FUDR ; [9] β-cyanoethyl phosphate prepared by the method of Tener (9) and used to phosphorylate 3'-acetyl-FUDR in the synthesis of FUDRP according to the procedure of Remy and Heidelberger (10) ; tetrahydrofolic acid and 5-methyl tetrahydrofolic acid prepared by Mr. P. P. K. Ho and Mr. J. Mangum, respectively, using previously described procedures (11, 12) ; and 5-fluorouracil, 5-diazouracil, 6-mercaptopurine ribonucleoside, and 5-iododeoxyuridine.[10]

Criteria for the selection of clinical material used in this study have been give in a previous paper of this series (13). Detailed hematological data on the present patients including course of therapy are presented in a later table.

Leukocytes were isolated by a method described previously (13), suspended in 3 vol of cold 0.05 M barbital buffer, pH 7.6, and homogenized in a Virtis "45" homogenizer at 45,000 rpm for 3 minutes. After centrifugation of the homogenate for 15 minutes at 10,900 × g, the sediment was discarded, and the supernatant fraction was used for the enzyme assays. Protein was determined by the biuret method with bovine serum albumin as the standard. Deoxycytidylate deaminase was determined by the method of Maley and Maley (14).

Assay for thymidylate synthetase. The assay used in this study, based upon the incorporation of HC¹⁴HO into thymidylate, is adapted from several procedures described previously (15–18). The reaction mixture contained in a total volume of 0.8 ml: 0.7 μmole of tetrahydrofolate, 1 μmole (10 μc) of C¹⁴-formaldehyde, 10 μmoles of MgCl₂, 1 μmole of EDTA, 0.5 μmole of dUMP, 5 to 15 mg of leukocyte protein, and 50 μmoles of Tris buffer, pH 7.5. The control mixture was identical except for the omission of dUMP. After incubation for 1 hour at 37° C, the mixture was chilled, and 1 μmole of carrier TMP was added, followed immediately by the addition of an equal volume of cold 10% trichloroacetic acid. Protein was removed by centrifugation, and the supernatant fluid was evaporated to dryness. The residue was dissolved in 0.5 ml of concentrated perchloric acid and heated for 1 hour at 100° C. The acid was neutralized with KOH, and insoluble potassium perchlorate was removed by centrifugation. The supernatant fraction was passed through a column 1 cm in diameter containing 3 cm of Dowex-1-formate over 3 cm of Dowex-50 (H⁺). The unadsorbed material and the effluent obtained by washing the column with water were pooled, concentrated by heating, and chromatographed on Whatman 1 paper using 1-butanol : water (86 : 14) as the solvent system. The

procedure resulted in the separation of uracil ($R_f = 0.31$) and thymine ($R_f = 0.52$). The concentration of thymine, after elution from the paper, was determined spectrophotometrically at 264 mμ with a millimolar extinction coefficient of 7.9. A 0.1-ml sample of the thymine-containing solution was dissolved in 2 ml of hydroxide of Hyamine, to which was added 10 ml of toluene, containing 0.01% POPOP and 0.4% PPO ; the radioactivity in the sample was determined at 950 v in a Packard liquid scintillation counter. All results were corrected for blanks (initial reaction mixture less dUMP) carried through the same procedure ; the blank corrections were never larger than 10% of the experimental values.

The spectrophotometric assay of Wahba and Friedkin (7), which is based upon the absorbancy change at 339 mμ associated with the conversion of N^5,N^{10}-methylene tetrahydrofolate to dihydrofolate, was used for the assay of the partially purified thymidylate synthetase.

Purification of thymidylate synthetase. Two hundred and fifty ml of blood was obtained from a patient with chronic myelocytic leukemia who had a leukocyte count of 178,000. All subsequent operations were carried out at 4° C. The isolated leukocytes (about 35 ml of packed cells) were homogenized in 100 ml of 0.05 M barbital buffer, pH 7.6, and the homogenate was centrifuged at 10,900 × g. One-third volume of 2% protamine sulfate was added to the supernatant fluid, and the precipitate was removed by centrifugation. The supernatant fluid was then brought to 40% saturation with solid ammonium sulfate (24.3 g per 100 ml of solution), and the resulting precipitate was discarded. The concentration of ammonium sulfate in the supernatant fraction was increased to 60% saturation by the addition of 13.2 g per 100 ml of solution. The 40 to 60% precipitate was collected by centrifugation, dissolved in 10 ml of 0.05 M barbital buffer, pH 7.6, and dialyzed for 2 hours against the same buffer. The above procedure resulted in a tenfold purification and a 40% recovery of the enzyme relative to the original homogenate.

RESULTS

Properties of thymidylate synthetase. With the HC¹⁴HO assay system, it was established that the rate of the reaction catalyzed by the leukocyte thymidylate synthetase was proportional to enzyme concentration and to time. The pH-activity curve showed a rather broad optimum with the maximum centered around pH 6.5 (Figure 1). In the leukocyte homogenate, the enzyme was stable at 4° C for 24 hours, and 50% of the activity was retained when a dialyzed 40 to 60% ammonium sulfate fraction was stored at − 20° C for 2 weeks.

A component study for Reaction 1 is presented in Table I. There is an absolute requirement for both substrates, dUMP and N^5,N^{10}-methylene

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[6] Microchemical Specialties Co., Berkeley, Calif.

[7] Packard Instrument Co., La Grange, Ill.

[8] Burroughs Wellcome & Co., Tuckahoe, N. Y.

[9] Kindly supplied by Dr. C. Heidelberger and Dr. D. Remy.
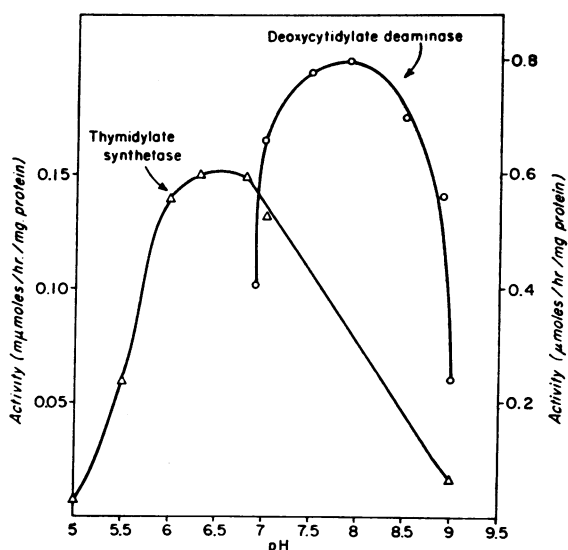
[10] Gifts of Dr. D. M. Donohue.

Fig. 1. pH ACTIVITY CURVE FOR THYMIDYLATE SYNTHETASE ($\triangle$, LEFT ORDINATE) AND DEOXYCYTIDYLATE DEAMINASE ($\bigcirc$, RIGHT ORDINATE).

tetrahydrofolate (the latter being generated from HCHO and tetrahydrofolate), and a partial requirement for Mg^{++} and for EDTA. No explanation can be offered at present for the function of the latter component. Substitution of 5-methyl tetrahydrofolate for N^5,N^{10}-methylene tetrahydrofolate, or of formate for formaldehyde, resulted in a complete loss of activity. The synthetase was also specific for dUMP, since UMP, deoxyuridine, or uracil could not replace the deoxyribonucleotide as the acceptor for the C_1 group. It should be noted that dCMP can serve as a substrate for thymidylate synthetase in crude preparations, since it is converted to dUMP via deoxycytidylate deaminase; this reaction will be discussed subsequently.

When 1 μmole of TMP was added to the incu-

bation mixture at the beginning of the reaction, rather than added later as a carrier, there was no synthesis of additional thymidylate, demonstrating that the enzyme is inhibited by its reaction product. In the presence of 1 μmole of thymine or thymidine, thymidylate synthetase was inhibited approximately 50%, whereas the addition of 0.5 μmole of TTP or 1 mg of DNA did not affect the rate of thymidylate formation.

When tested at 10^{-4} M, the following antimetabolites had no effect on thymidylate synthetase: aminopterin, 5-fluorouracil, 5-diazouracil, 5-iodo-deoxyuridine, 6-mercaptopurine, 6-thioguanine, and 6-mercaptopurine ribonucleoside. On the other hand, fluorinated pyrimidines markedly inhibited the leukocyte enzyme, confirming previous findings with thymidylate-synthesizing systems from other tissues (18). In Table II, FUDR and FUDRP are compared with respect to their ability to inhibit the leukocyte synthetase. The deoxyribonucleoside is not inhibitory even at 10^{-3} M, whereas the deoxyribonucleotide still inhibits the enzyme completely at 10^{-6} M and to 40% at 10^{-7} M. The presence of a deoxyribonucleoside kinase, capable of utilizing FUDR as a substrate, is also evident from the data in Table II. Thus, addition of 10^{-2} M ATP to the otherwise non-inhibitory FUDR at 10^{-4} M resulted in complete inhibition of enzyme activity. ATP at this level had no effect on the synthetase activity.

The interrelationship of thymidylate synthetase and dihydrofolic reductase has been outlined in Equation 2. The coupling of synthetase and re-

TABLE I

Component study for thymidylate synthetase

Component omitted	Thymidylate synthesized
	$m\mu moles$
None	1.9
Tetrahydrofolate	0
HCHO	0.02
dUMP*	0
Mg^{++}	1.0
EDTA	0.8

* Deoxyuridine-5'-phosphate.

TABLE II

Effect of FUDR and FUDRP on thymidylate synthetase

Inhibitor*	Concentration	Inhibition
	M	%
FUDR	10^{-3}	0
	10^{-4}	0
	10^{-5}	0
FUDR + ATP	10^{-4} 10^{-2}	100
FUDRP	10^{-4}	100
	10^{-5}	100
	10^{-6}	100
	10^{-7}	40
	10^{-8}	0

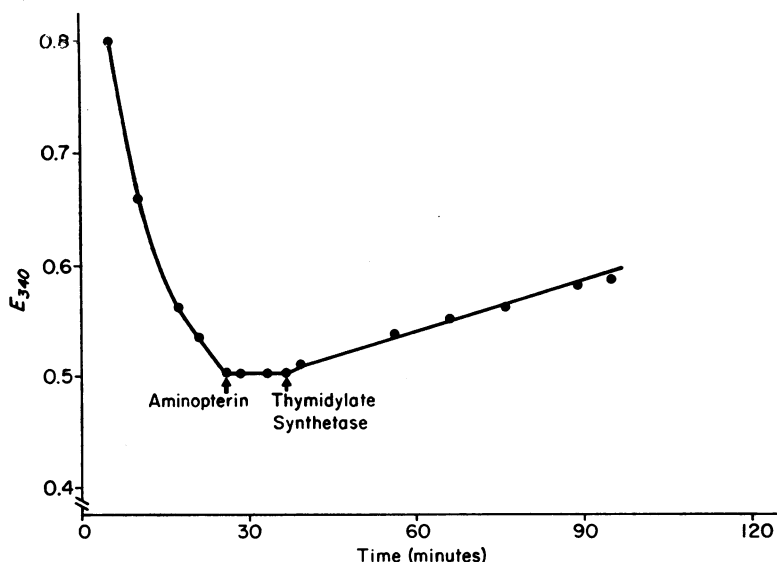* FUDR = fluorodeoxyuridine; FUDRP = fluorodeoxyuridine-5'-phosphate.

FIG. 2. SPECTROPHOTOMETRIC DEMONSTRATION OF THE INTERRELATIONSHIP OF DIHYDROFOLIC REDUCTASE AND THYMIDYLATE SYNTHETASE. The initial reaction mixture contained the following components in a cuvette of 1-cm optical path: 50 μmoles of Tris buffer, pH 7.5, 0.03 μmole of dihydrofolate, 0.08 μmole of TPNH, 1 μmole of HCHO, 10 μmoles of Mg^{++}, 1 μmole of EDTA, and 0.2 μmole of dUMP (deoxyuridine-5'-phosphate) in a volume of 1 ml. The optical blank was identical except for the omission of TPNH. The reaction was initiated by adding 2 mg of purified leukocyte dihydrofolic reductase (free from thymidylate synthetase) to each cuvette and measuring the absorbancy change at 340 mμ. At 25 minutes, 0.01 μmole of aminopterin was added, and at 35 minutes, 10 mg of purified leukocyte thymidylate synthetase was added. Absorbancy changes were also corrected for separate blanks in which dihydrofolate and dUMP, respectively, had been omitted.

ductase in the leukocyte could be demonstrated by the spectrophotometric experiment shown in Figure 2. The initial reaction mixture contained: purified leukocyte dihydrofolic reductase (containing no synthetase), dihydrofolate, TPNH, HCHO, and dUMP. An identical mixture, omitting TPNH, served as the optical blank. The initial decrease in absorbancy at 340 mμ was due both to the conversion of dihydrofolate to tetrahydrofolate and TPNH to TPN. Tetrahydrofolate then reacted chemically with excess HCHO to form N^5,N^{10}-methylene tetrahydrofolate, but this step is not accompanied by any appreciable change in absorbancy at 340 mμ. Aminopterin at 10^{-5} M was added (first arrow) to completely inhibit the reductase, and thus break the cycle. Upon adding purified thymidylate synthetase (second arrow), the increase in absorbancy indicated the regeneration of dihydrofolate from N^5,N^{10}-methylene tetrahydrofolate.

Properties of deoxycytidylate deaminase. In leukocyte lysates, the deamination of deoxycytidylate via Reaction 3 was found to be linear with time and enzyme concentration until about 60%

TABLE III

Deamination of cytosine derivatives

Substrate*	Activity
	$\mu moles/hr/mg\ protein$
Cytosine	0
Cytidine	0.8
Cytidine-2',3'-cyclic phosphate	0
CMP	0
CDP	0
CTP	0
Deoxycytidine	1.1
5-Methyl deoxycytidine	0.4
dCMP	0.6
dCDP	0
dCTP	0

* CMP, CDP, and CTP = cytidine mono-, di-, and triphosphate; dCMP, dCDP, and dCTP = deoxycytidine mono-, di-, and triphosphate.

of the substrate had disappeared. The pH optimum for this enzyme was 8.0, as shown also in Figure 1. When dCMP was deaminated, the product was identified as dUMP by paper chromatography and by its ability to serve as a substrate in the thymidylate synthetase reaction.

The specificity of the deaminase system for various cytosine derivatives is summarized in Table III. In addition to the deoxyribonucleotide (dCMP), deamination occurred with the deoxyribonucleoside (deoxycytidine), the 5-methyl deoxyribonucleoside, and the ribonucleoside (cytidine). Neither the free base (cytosine) nor the various ribonucleotides [CMP, CDP, CTP, and 2′,3′-(cyc)-CMP] were deaminated.

The mean level of deoxycytidylate deaminase in the leukocytes of ten normal subjects and eight patients with leukemia (acute, chronic myelocytic, and chronic lymphocytic) was 0.5 μmole per hour per mg of protein (range, 0.3 to 1.1). There was no statistically significant difference in the level of this enzyme between normal and leukemic cells.

Levels of thymidylate synthetase in normal and leukemic leukocytes. The levels of thymidylate synthetase were measured in the leukocytes of 11 normal subjects and 27 patients with leukemia. The results, accompanied by pertinent clinical data for each patient, are tabulated in Table IV. The enzyme was found in the cells of chronic myelocytic leukemia and acute leukemia, but it could not be detected in chronic lymphocytic leukemia or in normal cells. In chronic myelocytic leukemia, thymidylate synthetase was found in all 12 patients who were in relapse; the mean level was 0.19 ± 0.02 mμmole per hour per mg protein [11]

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[11] The extremely low level of thymidylate synthetase in the leukocyte becomes evident when this value is compared in the same units with the formate-activating enzyme (960 mμmoles per hour per mg protein) or lactic dehydrogenase (145,000 mμmoles per hour per mg protein).

TABLE IV

Levels of thymidylate synthetase in leukemia patients

Patient	Diagnosis*	Therapy	Leukocyte count	Percentage of mitotic cells (myelocytes, promyelocytes, blasts)	Thymidylate synthetase
			/ml	%	mμmoles/hr/mg protein
1	CML	None	70,000		0.25
2	CML	Busulfan	73,000		0.13
3	CML	X ray	111,000	30	0.18
4	CML	Busulfan	120,000	42	0.15
5	CML	None	35,000	35	0.34
6	CML	None	250,000	18	0.29
7	CML	None	45,000	9	0.10
8	CML	Busulfan	68,000	16	0.13
9	CML	None	40,000	19	0.21
10	CML	Colcemid	14,000	63	0.23
11	CML	Busulfan	175,000	51	0.15
12	CML	None	70,000	22	c. 0.06
13	ALL	6-Mercaptopurine	86,000	100	0
14	AML	None	82,000	97	0
15	ALL	None	17,000	39	0
16	AML	6-Mercaptopurine plus prednisone	52,000	93	0.07
17	AML	None	27,000	18	0.01
18	AML	6-Mercaptopurine	150,000	96	0.06
19	ALL	6-Mercaptopurine plus prednisone	103,000	99	0.15
20	CLL	None	44,000		0
21	CLL	Chlorambucil	62,000		0
22	CLL	None	200,000		0
23	CLL	None	35,000		0
24	CLL	Chlorambucil	40,000		0
25	CLL	Chlorambucil	28,000		0
26	CLL	None	90,000		0
27	CLL	None	43,000		0

* CML = chronic myelocytic leukemia; ALL = acute lymphocytic leukemia; AML = acute myelocytic leukemia; CLL = chronic lymphocytic leukemia.

with a range of 0.06 to 0.34. Levels of thymidyl-
ate synthetase comparable to those occurring in
chronic myelocytic leukemia cells have also been
observed in two patients with monocytic leukemia.
Of considerable interest is the additional finding
that two patients with chronic myelocytic leuke-
mia, who were in an acute crisis, showed greatly
elevated synthetase levels, i.e., 0.56 and 1.1
mμmoles per hour per mg protein, respectively.

In two additional patients (not listed in Table
IV), thymidylate synthetase activity was deter-
mined before and during therapy. The pretreat-
ment levels of the enzyme were 0.12 and 0.11
mμmole per hour per mg protein, respectively, and
the leukocyte counts were 163,000 and 45,000.
Upon treatment with busulfan and demecolcin,
the leukocyte counts decreased to less than 20,000,
and there was a proportional decrease in the per-
centage of immature cells. Concomitantly, the
synthetase level decreased and ultimately could
not be detected (value < 0.02 mμmole per hour
per mg protein). On the other hand, periodic as-
says performed on untreated patients, or patients
not responding to treatment, indicated that the
enzyme level did not fluctuate by more than
± 30%.

Although we had reported in a preliminary com-
munication (2) that thymidylate synthetase could
not be found in acute leukemic leukocytes, the
present studies using C^{14}-formaldehyde of a
higher specific activity have shown that a low
level of thymidylate synthetase (0.07 mμmole per
hour per mg protein) could be detected in four out
of the seven acute leukemic patients recorded in
Table IV. There was no apparent correlation be-
tween patients with the detectable enzyme levels
with the type of acute leukemia, percentage of
blasts, or therapy.

Thymidylate synthetase could not be detected
in the cells of 12 normal subjects or of eight pa-
tients with chronic lymphocytic leukemia. Fur-
thermore, no synthetase activity could be detected
in purified fractions from these cells that might
be expected to contain the enzyme. For example,
the leukocytes from 8 U of normal blood were
pooled and fractionated according to the proce-
dure used for the purification of thymidylate
synthetase. Both the crude lysate and the 40 to
60% ammonium sulfate fraction were devoid of
any measurable amount of synthetase.

Thymidylate synthetase was also found in two
nonleukemic patients with marked leukocytosis.
One patient had a leukemoid reaction secondary to
carcinoma, and the second had infectious mononu-
cleosis with a leukocyte count of 27,000. The
levels of thymidylate synthetase were 0.06 and
0.15 mμmole per hour per mg protein, respec-
tively; these activities fall within the range of
chronic myelocytic leukemia cells.

DISCUSSION

Winzler, Williams, and Best (19), working
with intact leukocytes, have reported that the
synthesis of thymidylate, as measured by the in-
corporation of C^{14}-formate or C^{14}-formaldehyde
into DNA, is inhibited by folic acid antagonists.
Since this inhibition does not occur with the par-
tially purified synthetase, which catalyzes Reac-
tion 1, it is evident that the antagonists are in-
hibiting dihydrofolic reductase, whose relation-
ship to the "thymidylate synthesis cycle" is out-
lined in Equation 2. Thymidylate synthetase
from the leukocyte, which catalyzes the transfer
of a C_1 unit from N^5,N^{10}-methylene tetrahydro-
folate to deoxyuridylate to yield thymidylate and
dihydrofolate, requires Mg^{++} for maximal ac-
tivity, has a pH optimum at 6.7, and is strongly
inhibited by FUDRP.

Inhibition of thymidylate synthetase has been
demonstrated with FUDRP, the deoxyribonucleo-
tide of fluorouracil; the deoxyribonucleoside,
FUDR, is ineffective. The latter compound, how-
ever, may merit attention in the treatment of
chronic myelocytic leukemia, since it is more read-
ily available than FUDRP and, being nonphos-
phorylated, passes through cell membranes easily
and can be phosphorylated within the cell to yield
FUDRP. The enzymatic phosphorylation of
FUDR, and an alternate pathway for the synthe-
sis of FUDRP from fluorouracil, have been stud-
ied in ascites tumor cells by Reichard (20).

The extremely low level of thymidylate synthe-
tase in the leukocyte presents some difficulties in
its detection, but this has been minimized by the
use of a C^{14}-labeled substrate having a high speci-
fic activity. The fact that thymidylate synthetase
activity occurs at a level two to three times lower
than that of other relevant enzymes which have
been studied, e.g., dihydroorotic dehydrogenase
(21) and dihydrofolic reductase (13), supports

the suggestion (22) that the reaction catalyzed by this enzyme is probably the rate-limiting step in the biosynthesis of DNA. It has been found previously that when thymine-2-C^{14} was added to suspensions of leukemic leukocytes, 91% of the labeled material was recovered in DNA, and none was found in the acid-soluble nucleotide pool (23). The administration of other labeled purine and pyrimidine precursors, however, resulted in the labeling of acid-soluble nucleotides. This would suggest that in the leukocyte the pool of free thymidylate is very small and that newly synthesized TMP is converted rapidly to the triphosphate and thence incorporated into DNA via the DNA-polymerase reaction; this suggestion has been substantiated recently by the findings of Bianchi (3).

Information about cellular proliferative activity obtained by cytological and cytochemical techniques in the intact leukocyte, as well as thymidylate synthetase and dihydrofolic reductase levels, is summarized in Table V. Good agreement exists among the various criteria applied to cellular proliferative activity. Chronic myelocytic leukemic cells have the highest rate of thymidylate synthesis (23–25) as judged from the incorporation of labeled precursors (formate, formaldehyde, or thymidine). Similarly, we have found the highest levels of thymidylate synthetase activity in these cells. The mitotic index of chronic myelocytic leukemia bone marrow is also higher than in acute leukemia, chronic lymphocytic leukemia, or in normal cells (26–28).

Acute leukemia appears to be heterogeneous inasmuch as the leukocytes of some, but not all, of the patients a) incorporate C^{14}-formaldehyde (24, 27) and H^3-thymidine into DNA and b) contain thymidylate synthetase. Chronic lymphocytic leukemic leukocytes and leukocytes from

normal subjects do not incorporate C^{14}-formaldehyde into DNA, and only a small percentage of the cells incorporate H^3-thymidine into DNA. Thymidylate synthetase is not detectable under the conditions of the present assay in either of the latter cell types. In addition to being metabolically interrelated, dihydrofolic reductase and thymidylate synthetase overlap in their cytologic distribution.

The finding that the level of deoxycytidylate deaminase is the same in both normal and leukemic leukocytes suggests that leukemic cells are not characterized by a uniform increase in all of the various enzymes involved in nucleic acid metabolism. Because of the recent use of halogenated derivatives of cytosine in the chemotherapy of tumors (29), the presence of this deaminase in the leukocyte offers a pathway for the conversion of these agents into the corresponding halogenated uracil derivatives, which may act as inhibitors of enzyme systems or be incorporated into DNA.

Although thymidylate synthetase has been detected in chronic myelocytic leukemic cells and in some acute leukemic cells, but not in chronic lymphocytic leukemic cells or in normal leukocytes, this enzyme is not unique to leukemia, since similar changes are found in infectious mononucleosis. It is likely that the presence of the enzyme is a reflection of the fact that in certain types of leukemia, as well as in infectious mononucleosis, the immature cells are characterized by an increased capacity for DNA synthesis.

SUMMARY

Thymidylate synthetase, which catalyzes the reaction, deoxyuridylate + N^5,N^{10}-methylene tetrahydrofolate → thymidylate + dihydrofolate, has been partially purified from leukocytes. The en-

TABLE V

Criteria of proliferative activity in leukocytes

Cell type	Mitotic activity		Incorporation into DNA thymidylate		Thymidylate synthetase	Dihydrofolic reductase
	Marrow	Blood	HC^{14}OOH or HC^{14}HO	H^3 thymidine		
Normal	+	0	0	0	0	±
Chronic lymphocytic leukemia	0	0	0	+	0	+
Chronic myelocytic leukemia	++++	++	++++	++++	++++	+++
Acute leukemia	++	+	+	++	+	++++

zymic reaction is assayed by means of the tetra-hydrofolate- and deoxyuridylate-dependent incorporation of $HC^{14}HO$ into thymidylate. There is also a partial requirement for Mg^{++} in the reaction. Deoxycytidylate can function as a substrate, but only after conversion to deoxyuridylate via a deaminase present in leukocytes. The synthetase has a pH optimum at 6.5 and is inhibited by 5-fluorodeoxyuridylate and by the reaction product, thymidylate. The continual synthesis of thymidylate involves a cyclic process in which the primary synthetase reaction is coupled with dihydrofolic reductase. The latter enzyme regenerates tetrahydrofolate, which is converted ultimately to the N^5,N^{10}-methylene derivative.

The level of thymidylate synthetase has been determined in the leukocytes of 12 normal subjects and 27 patients with leukemia. In chronic myelocytic leukemia, the mean level was 0.19 mμmole per hour per mg protein, and in four out of seven acute leukemia patients, the level was 0.07 mμmole per hour per mg protein. No activity could be detected in the cells from eight patients with chronic lymphocytic leukemia or from 12 normal subjects.

ACKNOWLEDGMENTS

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