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EEFECT OF 5-IODO-2′-DEOXYURIDINE ON THE BIOSYNTHESIS OF PHOSPHORYLATED DERIVATIVES OF THYMIDINE*

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Abstract—A study was made of the effect of 5-iodo-2′-deoxyuridine (IUDR) on the formation and utilization of phosphorylated derivatives of thymidine, as well as on the formation of DNA-thymidine, in various murine and human neoplastic tissues. The decreased incorporation, in the presence of IUDR, of the incorporation of ^{14}C-formate and ^{3}H-thymidine into DNA-thymine is a reflection of an inhibition of the utilization of thymidine, thymidylic acid or thymidine triphosphate, presumably by IUDR or the corresponding phosphorylated derivatives of IUDR. It is to be noted that the specific metabolic site primarily affected in the various tissues studied is a characteristic of the individual tissue. Thus, DNA-polymerase was primarily inhibited in murine Ehrlich ascites carcinoma and in human chronic granulocytic and acute monocytic leukemias, whereas studies with murine L5178Y leukemia cells, using ^{3}H-thymidine and ^{14}C-formate showed a prime blockade of thymidine kinase and thymidylic acid kinase, respectively. Confirmation of the inhibition of thymidine kinase was obtained with a cell-free extract of the L5178Y cells; however, with normal calf thymus, thymidylic acid kinase, but not thymidine kinase, was inhibited primarily. The apparent resistance to IUDR of the Walker carcinosarcoma 256 of the rat, *in vivo*, as well as of a patient with acute monocytic leukemia, could be explained by an inadequate dosage regimen, since marked inhibition of the biosynthesis of DNA by these tissues could be demonstrated *in vitro*.

INTRODUCTION

5-IODO-2′-DEOXYURIDINE (IUDR)[1] has been reported previously to be an effective competitive antagonist of the utilization of thymidine (TDR) for the biosynthesis of the thymidylic acid (TMP) portion of deoxyribonucleic acid (DNA).[1–6] Through a limitation on the use of thymidine derivatives, formed from either orotic acid or formate, the appearance of these precursors in DNA-thymine was inhibited by IUDR.[1,2] Thus, both the exogenous and the *de novo*-pathways concerned with the formation of phosphorylated derivatives of TDR were affected by IUDR (or its derivatives). This analog of thymidine inhibited the reproduction of mammalian[4–8] as well as of bacterial cells.[1] Although it had been demonstrated that IUDR can be incorporated extensively into the DNA-polymer,[3–11] it had not been established that inhibition of cellular reproduction is causally related to this observed biochemical

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event. The present report indicates that the decreased incorporation of precursors into DNA-thymine is a result of a primary inhibition of thymidine kinase, thymidylic acid (TMP) kinase, or DNA-polymerase, the specific site of the inhibition being characteristic of the cell type.

MATERIALS AND METHODS

Preparation of cell suspensions

The preparation of the Ehrlich ascites carcinoma cells has been described previously,[2] with the modification in some experiments of the use of Krebs 111 buffer,[12] in place of Chamber's solution.

The lymphoblastic leukemia cells (L5178Y) were maintained in AKR × DBA/2 F_1-hybrid mice and the preparation of cell suspensions was carried out in a manner identical to that used for the Ehrlich ascites cells.[2] Walker carcinosarcoma 256* was maintained in male weanling albino rats and 10 days following inoculation the tumor was removed, homogenized in Krebs 111 buffer, centrifuged, and resuspended in 5 vols of the buffer. Normal calf thymus was homogenized in Krebs 111 buffer and a suspension (25%) was prepared as described above.

Preparations of white blood cells from human leukemia patients were made by the method described by Walford *et al.*[13] Heparinized whole blood from leukemic patients was mixed with 0·25 vol of 6% dextran and allowed to stand at an angle of 40° at room temperature for 40 min; the upper layer composed of white blood cells was removed, centrifuged, washed with a solution of sodium chloride (0·9%) (henceforth referred to as "saline") and resuspended in 3 vols of Krebs 111 buffer.

Preparation of solutions

The sodium ^{14}C-formate and ^{3}H-thymidine used had specific activities of 3 μc per μmole and 50 μc per μmole, respectively. 5-Iodo-2′-deoxyuridine was dissolved either in Krebs 111 buffer (pH 7·2) or in water and the pH adjusted to about 7·5; the final concentration of the solution used was 5 μmoles/ml.

Incubation conditions

The reaction mixtures consisted of packed cells (0·25 ml), horse serum (0·2 ml), 5-iodo-2′-deoxyuridine in the indicated amount, ^{3}H-thymidine (5 μc) or ^{14}C-formate (3 μc) and Totter's modification of Chamber's solution or Krebs 111 buffer: final volume, 2·3 ml. The incubations were conducted in triplicate in 20-ml beakers under an atmosphere of air, using a Dubnoff metabolic shaker at 37°; the beakers were agitated at 90 cycles/min for varying periods of times up to 2 hr. Following the incubation period the cells were separated by centrifugation and washed two or three times with saline prior to freezing. For serial time studies, the reactions were stopped at the appropriate time by the addition of trichloroacetic acid (TCA) (100%, 0·2 ml).

Preparation of cell-free extracts of mouse leukemia cells

Cells were harvested 6 days following the inoculation of AKR × DBA/2 F_1-hybrid mice with L5178Y ascites cells; after centrifugation, the cells were washed once with saline. Packed cells (3 ml), Tris buffer (0·05 M, pH 7·9) and glass beads (200 μ, 7 ml) were agitated for 20 sec in a Nossal vibrator. Following filtration through glass wool the filtrate was centrifuged at 10,000 **g** for 10 min. Both the composition of

* Rats bearing this tumor were kindly provided by the Research Laboratories of the Upjohn Company, Kalamazoo, Michigan.

the incubation mixture and the conditions used were similar to those described by Weissman *et al.*[14] The reaction mixture (1·2 ml) contained $MgCl_2$ (5·5 μmoles), ATP (110 μmoles), Tris buffer (pH 7·9, 110 μmoles), ^{3}H-TDR (5 μc, 0·01 μ mole) and various levels of IUDR. The reaction was terminated, after incubation for 90 min at 37°, by the addition of TCA and was analysed as described below.

Isolation and separation of phosphorylated derivatives of thymidine

All reactions were performed at 0 to 4 °C. The washed or frozen cells were suspended in ice-cold TCA (5%, 5 to 10 ml) for 20 min. Following centrifugation, the TCA-extraction was repeated and the supernatant fractions were combined. The TCA was removed by ether-extraction and the residual ether was removed by bubbling nitrogen through the solution. The pH was adjusted to 7·5 ± 0·5 with NaOH (0·2 N) and the nucleotides were adsorbed on a DEAE-cellulose column (8 × 1 cm). Separation of TDR, TMP, thymidine diphosphate (TDP) and thymidine triphosphate (TTP) fractions was performed by the method of Weissman *et al.*[14]

Determination of total radioactivity in each fraction

Whereas complete separation of excess ^{3}H-TDR from the nucleotide fractions could be readily achieved, difficulty was encountered in attaining the removal of excess ^{14}C-formate. Hence, in studies with ^{3}H-TDR, the total radioactivity in each nucleotide fraction was measured in a windowless flow counter (following the plating of appropriate aliquots in the center of a stainless steel planchet) or in a Packard liquid scintillometer. In all studies with ^{14}C-formate, because of the known contamination with non-thymine radioactive substances in each of the separated fractions composed of the individual thymine nucleotides, the following procedure was adopted. Subsequent to the addition of thymine (1 μmole) to each fraction, the solution was evaporated to dryness and the residue was digested with perchloric acid,[15] in order to hydrolyse the thymine-containing nucleotides to free thymine. Thymine was separated from other bases and from ^{14}C-formate by paper chromatography in the *iso*propanol-HCl system;[16] this separation was followed by re-chromatography in the butanol–ammonia system.[17] The concentration of thymine was determined in the Beckman u.v.-spectrophotometer and the radioactivity was measured in the manner described above. This information permitted the calculation of the total amount of radioactivity in each fraction associated with the thymine-containing nucleotides. The specific activity of isolated DNA-thymine was determined in the manner described previously.[2]

RESULTS AND DISCUSSION

Effect of IUDR on the Biosynthesis of Phosphorylated Derivatives of Thymidine

I. *Mouse tumors*

A. *Lymphoma* L5178Y *cells* in vitro: (i) *With* ^{3}H-*thymidine as the precursor.* In Table 1 are shown the results obtained from pre-incubation of L5178Y cells with IUDR for 15 min prior to the addition of ^{3}H-TDR. A seven-fold increase in the specific activity of DNA was observed in the presence or absence of IUDR when the incubation was prolonged from 0·5 hr to 2·0 hr, although at both intervals of time there was an identical degree of inhibition (78 per cent) by IUDR of the utilization of ^{3}H-TDR for the biosynthesis of DNA. In the presence of the analog, both at 0·5 and at 2 hr, there was a decrease of about 94 per cent in the amount of radioactivity in

the combined nucleotide pools. The decreased incorporation of ^{3}H-TDR into DNA-thymine appears to be a result of an inhibition which occurred prior to the formation of TMP. Two possible mechanisms of inhibition include a block by IUDR of TDR kinase or of the mechanism of transport of ^{3}H-TDR into the cell. Support for the former hypothesis is described below (see section B).

TABLE 1. THE EFFECT OF PRE-INCUBATION OF IUDR ON THE UTILIZATION OF $\pm^3$H-TDR FOR THE BIOSYNTHESIS OF TMP, TDP, TTP AND DNA-THYMINE BY MURINE L5178Y LEUKEMIC CELLS *in vitro**

Analog†	Time of incubation (hr)	Distribution of radioactivity			
		TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-T (counts/min per mg DNA)
—	0·5	13,000	51,400	95,600	2280
IUDR	0·5	1800	830	6200	490
—	2·0	21,500	36,400	104,000	15,700
IUDR	2·0	650	2360	6770	3400

* Details of incubation conditions are described in the text.
† Cells were pre-incubated for 15 min with IUDR (2 μmoles per ml) prior to the addition of ^{3}H-TDR (0·04 μmoles per ml).

Because TDR *per se* is not on the pathway for the biosynthesis *de novo* of TMP and since it had been shown previously[2] that IUDR inhibits the appearance of either orotic acid or formate in the thymine of DNA, but not the utilization of orotic acid for the biosynthesis of DNA-cytosine or of RNA-pyrimidines, it was apparent that IUDR must exert its inhibitory effect at another site, presumably after the formation of TMP.

In a second experiment it was hoped to create conditions whereby the various phosphorylated derivatives of TDR would have a better opportunity of being formed prior to the development of the inhibitory effect of IUDR. In Table 2 are shown the results obtained when IUDR was added to the reaction mixtures before, after or simultaneous with the inclusion of ^{3}H-TDR; however, there was no significant accumulation of radioactivity in any of the nucleotide pools regardless of the time of addition of IUDR. The variation in the absolute amount of radioactivity in the

TABLE 2. EFFECT OF TIME OF ADDITION OF ^{3}H-TDR IN RELATION TO IUDR ON THE FORMATION OF TMP, TDP, TTP AND DNA-THYMINE BY L5178Y CELLS *in vitro**

Time of addition of ^{3}H-TDR in relation to IUDR	Distribution of radioactivity			
	TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-T (% inhibition)
No IUDR	23,000	12,000	7700	—
15 min before	290	700	none	39
Simultaneously	1200	none	none	77
15 min after	150	none	110	85

* Details of incubation conditions are described in the text.

total acid-soluble pool of the control cells, as well as the different distribution of radioactivity among the nucleotides, as compared with the findings obtained in the previous experiment, may be a reflection of a variation in metabolic activity of different batches of cells. It was found that the extent of inhibition of the incorporation of ^{3}H-TDR into DNA-thymine varied inversely with the time of addition of IUDR. Thus, the largest incorporation of radioactive TDR into DNA-thymine was observed when the addition of IUDR followed pre-incubation with ^{3}H-TDR for 15 min. It appeared that the nucleotide fractions were rapidly formed during the 15-min interval prior to the addition of IUDR, and that these radioactive pools, formed in the absence of IUDR, were utilized subsequently for the formation of DNA-thymine. Accordingly, the data suggested that the utilization of ^{3}H-TDR for the biosynthesis of DNA-thymine by L5178Y cells *in vitro* is blocked by IUDR primarily at a stage prior to the formation of TMP.

(ii) *With ^{14}C-formate as the precursor.* For the reasons described above a study was made of the effect of IUDR on the formation of thymine-nucleotides derived from the *de novo*-pathway in which TDR *per se* does *not* participate. In Table 3 is shown the effect of IUDR on the utilization of ^{14}C-formate for the biosynthesis of TMP, TDP, TTP and DNA-thymine by L5178Y cells *in vitro*.

TABLE 3. THE EFFECT OF IUDR ON THE UTILIZATION OF ^{14}C-FORMATE FOR THE BIOSYNTHESIS OF TMP, TDP, TTP AND DNA-THYMINE BY L5178Y LEUKEMIC CELLS *in vitro**

Concentration of IUDR (μmoles/ml)	Distribution of radioactivity			
	TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-thymine (counts/min per mg DNA)
none	2900	5900	12,400	17,000
0·2	1480	2360	4700	7800
2·0	1880	675	1800	5100

* Details of incubation conditions are described in the text.

In agreement with earlier studies,[2] marked inhibition of the utilization of formate for the biosynthesis of DNA-thymine was observed. There was no inhibition in the conversion of TDP to TTP, since in the presence or absence of IUDR there was about a two-fold increase in the amount of radioactivity in the TTP fraction, as compared to that in TDP. Comparison of the TMP and TDP fractions revealed a 100 per cent increase in the amount of radioactivity in the TDP-fraction of the control cells, a 50 per cent increase in the presence of the lower concentration of IUDR, but *no* increase in the TDP fraction at the higher level of IUDR. In fact, in the presence of the higher concentration of IUDR, the amount of radioactivity of the TDP fraction *decreased* to 36 per cent of that present in the TMP fraction.

Although the kinase concerned with the phosphorylation of TMP to a higher level of phosphorylation appears to be a major site of inhibition by IUDR (or more probably by 5-iodo-2′-deoxyuridine 5′-phosphate), it would appear that other sites may be affected as well. For example, although *no* inhibition of thymidylic acid kinase was observed in the presence of the lower levels of IUDR (Table 3), there was a marked decrease in the specific activity of DNA-thymine.

This latter inhibition appears to be a reflection of the decreased *formation* of TMP. Although a reproducibly larger amount of radioactivity appeared in the

TMP fraction in the presence of the higher level of IUDR, this probably reflects an inhbition of TMP kinase. At the lower level of IUDR no inhibition of TMP kinase was observed; hence, the relative distribution of radioactivity among the TMP, TDP and TTP fractions was similar to that of the control. Thus, almost three times as much radioactivity is observed in the TDP and TTP fractions derived from cells incubated with the *lower* concentration of IUDR, as compared to those derived from the *higher* level of IUDR. Whether the decreased formation of TMP by L5178Y cells in the presence of IUDR is related causally to an inhibition of thymidylate synthetase or of a more obscure metabolic reaction is under investigation.

B. *Cell-free extract of* L5178Y *leukemia cells*: (i) *With* ^{3}H-*thymidine as the precursor.* A cell-free extract of mouse L5178Y cells was incubated with ^{3}H-thymidine in the presence of various amounts of IUDR (Table 4); at a molar ratio of IUDR to TDR

TABLE 4. EFFECT OF IUDR ON THE PHOSPHORYLATION OF ^{3}H-TDR IN A CELL-FREE PREPARATION OF MURINE LEUKEMIA L5178Y CELLS

Concentration of IUDR* (μmoles)	Thymidylic acid (Total counts/min)
none	7700
0·01	7000
0·1	5100
0·5	3300

* Concentration of IUDR in reaction mixture (1·2 ml) which contained thymidine (0·01 μmole, 0·5 μC). Details are described in text.

of 1 : 1, no decrease in the formation of TMP was observed; at ratios of 10 : 1 and 50 : 1, inhibition was observed to the extent of 35 and 57 per cent, respectively. Thus, the decreased utilization of ^{3}H-TDR in the presence of IUDR by the corresponding whole cell preparation described above may be explained by the observed inhibition of TDR kinase. Whether or not there is in addition, any effect on the mechanism of transport of TDR into cells was not investigated.

C. *Murine Ehrlich ascites carcinoma cells* in vitro. In contrast to the observations made with L5178Y cells, IUDR exerted no inhibition in the Ehrlich ascites carcinoma cells, with respect to either the transport of ^{3}H-TDR into the cells or its subsequent phosphorylation by thymidine kinase (Table 5). In addition, there was no inhbition

TABLE 5. THE EFFECT OF IUDR ON THE UTILIZATION OF ^{3}H-TDR FOR THE BIOSYNTHESIS OF TMP, TDP, TTP AND DNA-THYMINE BY MURINE EHRLICH ASCITES CELLS *in vitro*

	Distribution of radioactivity			
Analog	TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-T (counts/min per mg DNA)
none	5500	4500	3300	13,900
IUDR	6500	5000	6800	1800

* Details of incubation conditions are described in the text.

of the conversion of TMP to TDP or of TDP to TTP; rather, there appeared to be an accumulation of cold acid-soluble nucleotides, particularly at the triphosphate level. In agreement with previous studies, there was a marked reduction in the specific activity of DNA-thymine. Thus, the block of the utilization of TDR appears to be at the triphosphate level in these cells.

II. *Calf thymus*

It was of interest to determine at which site or sites inhibition of the utilization of ^{3}H-TDR occurred in normal cells, such as the lymphoid tissue of calf thymus. The results are shown in Table 6. Although there was a 50 per cent decrease in the formation of TMP in the presence of IUDR, a more marked inhibition was observed in the phosphorylation of TMP to TDP. Based on the relative amounts of radioactivity in the TMP and TDP fractions of the control, the amount of radioactivity in the TDP fraction derived from cells incubated with IUDR was only about 20 per cent of that which was obtained when thymidylic kinase was not inhibited. There was no inhibition of the phosphorylation of TDP to TTP or of the utilization of TTP for DNA-biosynthesis. The 80 per cent decrease observed in the specific activity of DNA-thymine is probably a reflection of the primary inhibition of TMP kinase, coupled with a less marked inhibition of either the uptake of TDR by the cells or of its initial phosphorylation to TMP.

TABLE 6. EFFECT OF IUDR ON THE UTILIZATION OF ^{3}H-TDR FOR THE BIOSYNTHESIS OF TMP, TDP, TTP AND DNA-THYMINE BY NORMAL CALF THYMUS *in vitro**

Analog	Distribution of radioactivity			
	TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-thymine (counts/min per mg DNA)
None	23,000	10,000	980	14,000
IUDR	11,500	1800	270	3000

* Details of incubation conditions are described in the text.

III. *Human leukemia cells*

A. *Chronic granulocytic leukemia cells.* Peripheral leukocytes obtained from two patients with chronic granulocytic leukemia were studied; the results, shown in Table 7, indicate that the pattern

TABLE 7. EFFECT OF IUDR ON THE UTILIZATION OF ^{3}H-TDR FOR THE BIOSYNTHESIS OF TMP, TDP, TTP AND DNA-THYMINE BY HUMAN CHRONIC GRANULOCYTIC LEUKEMIA CELLS *in vitro**

Case	Analog	Distribution of radioactivity			
		TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-thymine (counts/min per mg DNA)
1	None	7600	7500	7300	1400
	IUDR	6500	17,500	7200	140
2	None	1400	1300	1100	1400
	IUDR	2700	1400	3200	620

* Details of incubation conditions are described in the text.

of inhibition was similar to that seen with murine Ehrlich ascites carcinoma cells. The uptake of ^{3}H-TDR by the cells was not inhibited by IUDR, nor were its subsequent phosphorylations to TMP, TDP and TTP; however, there was marked interference with the polymerase reaction in which TTP is utilized for the biosynthesis of DNA. Thus, in the presence of IUDR an increase in the amount of radioactivity in the cold acid-soluble nucleotide pools was observed, as well as a marked decrease in the specific activity of DNA-thymine.

B. *Acute monocytic leukemia cells.* The effect of IUDR on the utilization of ^{14}C-formate and of ^{3}H-thymidine for the biosynthesis of the various thymine-containing nucleotides, as well as its effect on the formation of DNA-thymine, were studied in white blood cells derived from two patients with acute monocytic leukemia.

The results (see Table 8) indicate that the formation of TMP from ^{3}H-TDR by the leukocytes of patient 1 was of the same order as that observed with murine L5178Y

TABLE 8. EFFECT OF IUDR ON THE UTILIZATION OF ^{14}C-FORMATE AND ^{3}H-TDR FOR THE BIOSYNTHESIS OF TMP, TDP, TTP AND DNA-THYMINE BY HUMAN ACUTE MONOCYTIC LEUKEMIC CELLS *in vitro**

Patient	Precursor	Analog	Distribution of radioactivity			
			TMP (counts/min)	TDP (counts/min)	TTP (counts/min)	DNA-T (counts/min per mg DNA)
1	^{3}H-TDR	None	33,000	340	1100	2000
		IUDR	2700	910	1500	280
2	^{3}H-TDR	None	37,000	15,000	1000	4700
		IUDR	17,000	15,000	800	860
	^{14}C-formate	None	3200	2300	620	2800
		IUDR	1800	2000	770	230

* Details of incubation conditions are described in the text.

leukemic cells; however, in patient 2 the effect on the formation of TMP was much less marked. Although the pools of TMP derived from ^{3}H-TDR in the absence of IUDR, in patients 1 and 2, were of essentially the same size, there was a marked difference in the amount of radioactivity in the TDP fraction. In comparison with the TMP fraction, the radioactivity in the TTP fractions in either the presence or the absence of IUDR was relatively low. Although there was little difference in the size of the TTP fractions in the presence or absence of IUDR, there was a very marked decrease in the specific activities of the DNA-thymine.

Accordingly, several sites of inhibition by IUDR and its phosphorylated derivatives may be implicated in these cells: (1) a partial block in the formation of TMP derived from the *de novo*-pathway; (2) a partial block in either the transport of TDR into the cell or of TDR kinase (the exogenous pathway); and (3) inhibition of DNA polymerases. The last site would appear to be the most critical one in these cells with respect to the inhibition of the biosynthesis of DNA.

C. *Cells of other types of human leukemia.* The effect of IUDR on the utilization of ^{3}H-TDR for the formation of TMP, TDP, TTP and DNA-thymine, was examined in cells derived from one case each of chronic lymphocytic leukemia, leukosarcoma and plasma cell leukemia. In the presence of the analog a very marked inhibition in the conversion of TDR to TMP was observed with each type of cell, a finding which might

be attributed either to interference with the transport mechanism or to inhibition of TDR kinase. Very little radioactivity appeared in TDP, TTP or DNA, even in the absence of IUDR; nevertheless, the amount of ^{3}H-TDR which appeared in DNA in the presence of IUDR was markedly reduced.

Mechanism of "apparent" resistance to IUDR in vivo. In a previous study of the effect of IUDR on the growth of various neoplasms in experimental animals, the Walker carcinosarcoma 256 was reported not to be affected by this analog.[7] Accordingly, it was of interest to determine whether the failure to inhibit this tumor is attributable to an inherent resistance to the inhibitory effects of IUDR. An homogenate of this tumor was prepared and the effect of IUDR on the utilization of ^{14}C-formate and of ^{3}H-TDR for the biosynthesis of DNA-thymine was investigated. The results (shown in Table 9) indicate that the biosynthesis of DNA is markedly

TABLE 9. THE EFFECT OF IUDR ON THE UTILIZATION OF ^{14}C-FORMATE AND ^{3}H-TDR FOR THE BIOSYNTHESIS OF DNA-THYMINE BY WALKER CARCINOMA 256 CELLS *in vitro**

Metabolite	Analog	DNA-thymine (counts/min per unit DNA)
^{14}C-Formate	None	21,000
^{14}C-Formate	IUDR	4300
^{3}H-TDR	None	16,000
^{3}H-TDR	IUDR	7000

* Details of incubation conditions are described in the text.

* cpm in amount of DNA corresponding to 1 mg of TDR in Stumph reaction.

inhibited by IUDR. It is pertinent that neither during nor subsequent to treatment with IUDR were manifestations of toxicity observed in the rats bearing the Walker carcinosarcoma 256. Thus, the apparent resistance of this neoplasm to suppression by IUDR *in vivo* probably is attributable to an inadequate dosage regimen in this animal species.

Similarly, in a patient with acute monocytic leukemia, no beneficial effect was noted after a 36 hr intravenous infusion of IUDR, during which time the subject was given a total dose of 300 mg of IUDR per kg of body weight. After continuous intravenous infusion of IUDR for 16 hr, samples of the white blood cells of this patient were examined *in vitro* with respect to their ability to utilize ^{3}H-TDR for the biosynthesis of DNA-thymine in the presence of various levels of IUDR; the results are shown in Table 10. It is apparent that the extracellular concentrations of IUDR in this patient were inadequate to exert any significant inhibitory effect on the uptake of ^{3}H-TDR, since *in vitro* the addition of increasing amounts of IUDR resulted in marked inhibitions of the biosynthesis of DNA-thymine. It may be concluded, at least tentatively, that if an adequate regimen of dosage with IUDR could be given, which would permit inhibitory concentrations to be attained under *in vivo*-conditions, inhibition of leukemic cell proliferation should be possible. However, such levels of dosage with IUDR probably could not be administered without causing intolerable effects on normal cells, particularly those of the bone marrow.

Of pertinence, however, are studies by Calabresi[18] which have demonstrated the feasibility of *regional* protection with arterially infused thymidine, given in small amounts (4 to 8 mg/kg), while IUDR is administered (110–120 mg/kg) by intravenous infusion.

TABLE 10. COMPARISON OF THE ABILITY OF WHITE BLOOD CELLS DERIVED FROM A PATIENT WITH ACUTE MONOCYTIC LEUKEMIA BEFORE AND DURING TREATMENT WITH IUDR TO UTILIZE ^{3}H-TDR FOR THE BIOSYNTHESIS OF DNA-THYMINE IN THE PRESENCE AND ABSENCE OF IUDR*

Concentration of IUDR (μmole/ml)	Specific activity of DNA	
	Before treatment (counts/min per mg DNA)	During treatment (counts/min per unit DNA)
None	5600	4650
0·2	—	2800
0·8	—	1800
2·0	680	860

* Details of incubation conditions are described in the text.

* 1 DNA unit is that amount giving same colored reaction as 1 mg TDR in the Stumph reaction.

A third example of resistance to IUDR was observed in a patient with lympho- si ia; neoplastic cells from this individual utilized ^{3}H-TDR poorly for the bio- s sis of DNA-thymine. In view of this circumstance, it is difficult to evaluate the apparent lack of inhibitory effect of IUDR in this situation.

Comparison of kinase activities in various mammalian tissues. During the course of this study, the kinase activities of various tissues have been studied. Although no unequivocal conclusion may be drawn concerning the question as to whether or not TDP is an intermediate in the conversion of TMP to TTP,[14, 19] it is of interest that IUDR had *no* influence on the ratio of TDP to TTP. If TDP is an intermediate in the formation of TTP, then the corresponding phosphorylated derivative of IUDR, iododeoxyuridine diphosphate, exerts no inhibitory effect on TDP-kinase. Incubation of acute monocytic leukemia cells (Table 8, patient 2) with radioactive TDR or formate resulted in the formation of TDP and TTP in the ratio of 15 : 1 and 4 : 1, respectively. This suggests that if TDP were not on the direct pathway for the biosynthesis of TTP, then the latter compound is uniquely unstable in this cell population. Bianchi[20] has claimed that human tissue contains only small amounts of enzyme which phosphorylate TDR to TMP and that *all* human tissues convert TMP completely into TTP in less than 30 min. The results shown in Tables 7 and 8 do not support these conclusions. For example, the TMP derived from human acute monocytic leukemia cells which were incubated for 2 hr with ^{3}H-TDR represented in two patients 60 and 94 per cent, respectively the radioactivity of the combined TMP, TDP and TTP fractions, whereas the TMP fraction derived from ^{14}C-formate, 50 per cent. A possible explanation for the differences observed may be that Bianchi[20] derived his conclusions from studies of particle-free fractions, whereas the present studies utilized intact cells. There are many factors which affect the rate of utilization

of thymidine for the biosynthesis of DNA. Bond *et al.*[21] observed extensive labeling of DNA when cells from a patient with chronic granulocytic leukemia were incubated *in vitro* with ^{3}H-TDR, but in chronic lymphocytic leukemia and myeloma, less than 3 per cent of the cells took up the ^{3}H-TDR. These authors suggested that the cells with low uptake either have a low turnover rate or lack the ability to utilize TDR, or have an unusually long DNA-synthesizing time. Moreover, Craddock[22] found that the uptake of ^{3}H-TDR did not correspond with the degree of morphologic immaturity of the cell population. Thus, in myeloproliferative disorders the highest degree of labeling was observed in patients with a low percentage of myeloblasts, while lymphocytes from both acute and chronic lymphatic leukemia patients showed a low uptake of radioactivity, in contrast to the high degree of uptake by normal thoracic duct lymphocytes. In addition, the uptake was low in two cases of plasma cell in which a large number of blast cells were present. In acute myeloblastic leukemia it was found that 12 per cent of the cells in the peripheral blood were moderately labeled, a circumstance which indicated that although many cells were synthesizing DNA, the rate per cell was quite slow.

Mechanism of action of 5-iodo-2′-deoxyuridine. Thymidine monophosphate is the initial compound common to (a) the *de novo*-pathway for the biosynthesis of the thymine-component of DNA, via 2′-deoxyuridylic acid, and (b) the exogenous pathway, which is concerned with the utilization of thymidine derived either from dietary sources, or from the degradation of DNA, or from biosynthetic reactions of other cells or tissues. The appropriate kinases subsequently catalyze the phosphorylation of TMP to TDP and TTP, prior to polymerization into DNA.[23–28] It may be inferred that IUDR is converted to the corresponding mono-, di- and tri-phosphates, since IUDR has been shown to be incorporated into DNA[3–11], by replacement of TMP. Inhibition of a specific kinase probably is mediated by the appropriate phosphorylated analog, e.g. TMP by 5-iodo-2′-deoxyuridine 5′-monophosphate (IUDR-5′-P). This relationship is depicted in Fig. 1. Thus, the utilization

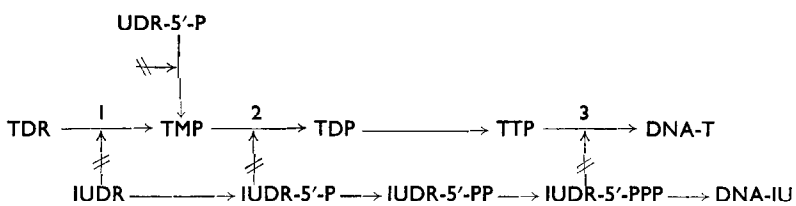

FIG. 1. Metabolic interrelationship of IUDR and precursors of DNA-thymine.

of thymidine or of formate for the biosynthesis of DNA-thymine by mouse L5178Y leukemia cells is blocked by the appropriate derivative of IUDR primarily at the thymidine kinase (reaction 1) and thymidylic acid kinase (reaction 2), respectively; whereas the utilization of these precursors for the biosynthesis of DNA-thymine in mouse Ehrlich ascites cells is blocked at a single site, DNA-polymerase (reaction 3). It is of interest that inhibition of the conversion of TDP to TTP was *not* observed in any of the tissues investigated.

An unexpected observation was the decreased formation of thymidylic acid via the *de novo*-pathway. Whether inhibition of thymidylate synthetase or of some other metabolic reaction is involved is under investigation.

The inhibitory effect of IUDR (or more probably its phosphorylated derivatives) is exerted at specific metabolic reaction sites, the primary site being related to the nature of the precursor as well as the species of cell.

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