Biochemical Pharmacology, Vol. 26, pp. 59–61. Pergamon Press, 1977. Printed in Great Britain.

SHORT COMMUNICATIONS

Utilization of ^3H from deoxyuridine and thymidine for synthesis of DNA and other macromolecules in various organs of the rat

(*Received* 21 *April* 1976; *accepted* 21 *May* 1976)

It has gradually become recognized that ^3H or ^{14}C from isotopically labeled thymidine can appear in macromolecular species other than DNA, among them glycogen [1], lipid [2], protein [2–4] and even RNA [5, 6]. Appearance of label in these molecules represents utilization of products of thymidine catabolism, the initial step being reversibly catalyzed by thymidine phosphorylase (EC 2.4.2.4, thymidine: orthophosphate deoxyribosyltransferase) or uridine phosphorylase (EC 2.4.2.3, uridine: orthophosphate ribosyltransferase), two different enzymes with overlapping substrate specificities [7–9].

Among the rat organs which we have examined, only those reported to have thymidine or uridine phosphorylase activities, the liver, intestinal mucosa, and bone marrow [10, 11], incorporated significant amounts of ^3H from deoxyuridine-[6-^3H] or thymidine[methyl-^3H] within 1 hr into an RNA fraction obtained by alkaline hydrolysis of acid precipitated tissue homogenates (see Table 1). In intestinal mucosa, the radioactivity in the RNA fraction was 15 per cent of that in the DNA when [^3H]deoxyuridine was injected and 2 per cent when [^3H]thymidine was injected. In adult rat liver, where the rate of cell proliferation is much lower than for intestinal mucosa, the radioactivity in the RNA fraction was five times that in DNA when the precursor was [^3H]deoxyuridine, and two times that in DNA when the precursor was [^3H]thymidine.

RNA and DNA were extracted separately by an adaptation [12] of the procedures of Schmidt and Thannhauser [13] and of Schneider [14] employing hydrolysis of the RNA in 1 N NaOH and extraction of the DNA with 5% trichloroacetic acid at 90°. DNA was measured by the method of Burton [15] with calf thymus DNA as the standard and radioactivity was measured in mixtures described by Patterson and Greene [16]. Deoxyuridine[6-^3H] (17 Ci/m-mole) and thymidine[methyl-^3H] (3 Ci/m-mole) were obtained from Schwarz/Mann, Orangeburg, N.Y. Rats were killed 1 hr after intraperitoneal injection of 50 μCi of the ^3H-precursor.

Significantly more radioactivity was in the RNA fraction of bone marrow when [^3H]thymidine was given than when [^3H]deoxyuridine was given. This was found for both hepatoma bearing (Table 1) and normal rats (H. Hopkins and J. Wakefield, unpublished observations). This result suggests that rat bone marrow contains thymidine phosphorylase and little or no uridine phosphorylase, or that bone marrow uridine phosphorylase has different substrate specificity than the liver or intestine enzymes. It should be noted in Table 1 that the ^3H activity in RNA fractions from liver, intestine, spleen and thymus did not differ for the two precursors.

The occurrence of ^3H from deoxyuridine in the RNA fraction of liver and intestinal mucosa is not surprising, since the uracil formed by the phosphorylase catalyzed reaction may be further metabolized to UMP either by consecutive reactions involving uridine phosphorylase and uridine kinase (EC 2.7.1.48, ATP: uridine 5′-phosphotransferase) or directly by a phosphoribosyltransferase [17]However, the radioactivity in the RNA fraction after [^3H]thymidine injection is surprisingly high, in view of the minor occurrence of thymine in RNA [5, 6] and the possibility that the thymine in RNA is formed by methylation after RNA synthesis. It is likely that the radioactivity observed in this RNA fraction after injection of [^3H]thymidine or [^3H]deoxyuridine resides in a macromolecule other than RNA, such as an alkali-solubilized, trichloroacetic acid-soluble protein or in lipid. Glycogen labeling [1] cannot be responsible for the radioactivity, since homogenization with the Polytron homogenizer (Kinematic GmbH., Lucerne, Switzerland) and the two extractions with 10% trichloroacetic acid remove all glycogen from liver samples prior to alkaline hydrolysis [18]. A protein determination [19] on the RNA fraction from liver indicated that approximately 25 per cent of the protein precipitated with cold 10% trichloroacetic acid is rendered soluble in this acid by incubation with 1 N NaOH for 1 hr at 37°. Extraction of lipid was not carried out prior to

Table 1. Incorporation of ^3H from deoxyuridine and thymidine into DNA and RNA fraction of rat organs and hepatoma 3924A

	^3H/mg DNA			
	Deoxyuridine[6-^3H]		Thymidine[methyl-^3H]	
	RNA fraction (dis./min)	DNA (dis./min)	RNA fraction (dis./min)	DNA (dis./min)
Liver	18,700 ± 480 (45)*	3,600 ± 210 (46)	16,100 ± 470 (3)	8,820 ± 1,470 (3)
Intestinal mucosa	4,180 ± 330 (44)	28,300 ± 2,260 (44)	3,560 ± 240 (3)	199,000 ± 1,390 (3)
Tibial bone marrow	740 ± 34 (13)†	33,100 ± 1,840 (13)	2,180 ± 970 (3)†	114,000 ± 8,670 (3)
Spleen	460 ± 12 (13)	6,160 ± 720 (13)	500 ± 25 (3)	39,400 ± 5,710 (3)
Thymus	210 ± 5 (46)	640 ± 26 (46)	190 ± 11 (3)	9,530 ± 450 (3)
Hepatoma 3924A	870 ± 59 (44)	14,800 ± 1,240 (45)		

* Number of rats comprising the mean.
† Means having the same footnote symbol differ significantly ($P < 0.01$).

hydrolysis of the RNA, since protein, RNA and DNA are all soluble to some extent in lipid solvents (see Ref. 12 for discussion). Therefore, lipid substances solubilized by 1 N alkali would be extracted along with the RNA. Schneider and Greco [2] observed ^3H in lipid, RNA and protein fractions from liver after injection of thymidine [methyl-^3H] and established that the labeling was from thymidine, not from radiolysis products. These workers located the ^3H in the glyceryl portion of glycerides and in serine, aspartic acid, glutamic acid, alanine and methionine of protein; the molecule responsible for the radioactivity in the RNA fraction was not identified by these workers. Further investigations will be necessary in order to identify the macromolecules which are responsible for the radioactivity in the RNA fractions obtained by our methods. For this reason we have used the term RNA fraction rather than RNA.

Incorporation of ^3H from [^3H]deoxyuridine into the DNA and RNA fractions of liver and intestinal mucosa represents competition for a single substrate by opposing pathways *in vivo*, regardless of whether the ^3H is in RNA or some other macromolecule in this fraction. Therefore, it was of interest to determine whether a decrease in utilization by the DNA synthetic pathway would be accompanied by increased incorporation in the RNA fraction. Rats given the LD_{10} dose of 5-fluorouracil (5-FU), 150 mg/kg body weight, exhibit 67 per cent inhibition of [^3H]deoxyuridine incorporation into liver DNA and greater than 95 per cent inhibition of incorporation into DNA of intestinal mucosa, spleen, thymus, tibial bone marrow and heptoma 3924A [20, 21]. In addition, enhanced incorporation of this precursor into DNA occurs at characteristic times for each organ during recovery from 5-FU toxicity. Inhibition of DNA synthesis by 5-FU resulted in increases of 60–80 per cent in radioactivity of RNA fractions of intestinal mucosa and bone marrow (compare Tables 1 and 2). Radioactivity in the RNA fractions of the other organs and hepatoma 3924A was not affected by inhibition of DNA synthesis. During recovery from the toxic effects of 5-FU, when DNA synthesis was enhanced, ^3H activity in the RNA fractions was increased 16 per cent in liver and 2- to 4-fold in other host organs but not in hepatoma 3924A. This increased incorporation into both DNA and the RNA fraction may reflect an increase in availability of the precursor to both catabolic and DNA synthetic pathways, or there may be increased utilization of the catabolic products during these periods of rapid cellular proliferation. In any event, there was no indication of decreased catabolism of [^3H]deoxyuridine during the periods of increased utilization for DNA synthesis. Extramedullary hematopoeises in spleen and liver after loss of bone marrow probably accounts for much of the enhanced [^3H]deoxyuridine incorporation into DNA for these organs during recovery after 5-FU administration (H. Hopkins, J. Wakefield, M. Stuart and W. Looney, unpublished observations).

There was greater utilization of thymidine than of deoxyuridine for DNA synthesis in all the organs (Table 1), related perhaps to the greater specificity of thymidine kinase (EC 2.7.1.2, ATP: thymidine 5′-phosphotransferase) for thymidine as substrate [22]. Although cellular proliferation in thymus is rapid [23], incorporation of the ^3H-precursors into DNA is low for this organ in comparison with the other rapidly proliferating organs. This observation was first made by Nygaard and Potter [24] using [^{14}C]thymidine and was attributed to differences in pool size for one or more of the intermediates between the injected precursor and DNA. Although differences in pool size may be a factor in this discrepancy, other factors such as cellular levels of thymidine kinase and the reported differences in kinetic properties of the enzyme purified from calf thymus [25] versus other sources should be more fully explored.

In hepatoma 3924A, the labeling index [26] and the radioactivity in DNA 1 hr after administration of [^3H]thymidine increase after treatment with 5-FU. Maximum values are reached approximately 24 hr after the drug is given. This result is consistent with the known action of 5-FU in inhibiting thymidylate synthetase, resulting in cellular arrest at the G_1-S boundary of the cell cycle [27]. However, normalization of the [^3H]thymidine in DNA to the labeling index for each respective tumor (Table 3) revealed that incorporation per labeled cell at 24 hr was not different from that during hr 1 after drug treatment although the size of intermediate pools would be expected to be smaller at 24 hr. It is possible that the amount of [^3H]thymidine which has entered the cell and is available for DNA synthesis does not differ for the three conditions in Table 3. A more rapid utilization of the [^3H]thymidylate phosphates in the absence of *de novo* thymidylate synthesis would then account for the 40 per cent increase in [^3H]thymidine in DNA when 5-FU treated tumors are compared with control (Table 3). In regenerating rat liver, cells which become labeled within 1 hr continue to incorporate [^3H]thymidine into DNA for 2 additional hr [28]. The decreasing availability of the labeled thymidine for DNA synthesis *in vivo* contrasts with that which is possible *in vitro* where the concentration of thymidine available to the cell can be held virtually constant during the labeling period. For cells in culture, the use of 5-FU or its deoxyriboside, 5-fluorodeoxyuridine, greatly enhances incorporation of thymidine or its analogs [29] into DNA.

Table 2. Incorporation of ^3H from deoxyuridine[6-^3H] into the RNA fraction of rat organs and hepatoma 3924A after perturbation of DNA synthesis with 5-FU

	^3H/mg DNA					
	DNA synthesis inhibited			DNA synthesis enhanced		
	Hours after 5-FU	RNA fraction (dis./min)	DNA dis./min)	Days after 5-FU	RNA fraction (dis./min)	DNA (dis./min)
Liver	1–36	18,600 ± 1,450 (10)*	1,330 ± 77 (11)	6	21,700 ± 580 (3)	35,200 ± 1,480 (3)
Intestinal mucosa	1–36	6,570 ± 520 (11)	1,000 ± 240 (11)	4	9,700 ± 2,840 (3)	202,000 ± 10,500 (3)
Tibial bone marrow	1–36	1,250 ± 200 (11)	3,500 ± 670 (11)	8	ND†	69,500 ± 25,500 (3)
Spleen	1–36	440 ± 27 (10)	280 ± 43 (10)	11	1,790 ± 590 (3)	69,700 ± 18,300 (3)
Thymus	1–36	210 ± 25 (11)	820 ± 25 (11)	9	825 ± 35 (3)	9,450 ± 3,200 (3)
Hepatoma 3924A	1–36	880 ± 92 (10)	1,070 ± 180 (11)	11	880 ± 140 (3)	19,300 ± 5,200 (3)

* Number of rats comprising the mean.
† Not determined.

Table 3. Comparison of 1-hr cell labeling index and [^{3}H]thymidine incorporation into DNA of Morris hepatoma 3924A for rats treated with 5-FU*

Hours after 5-FU (150 mg/kg)	No. of rats	Labeling index	[^{3}H]TdR incorporation (dis./min/mg DNA)	Incorporation/ L.I. 10^4
1	5	16.9†	296,800‡§	1.76‡
24	5	27.7†‖	504,400†‡	1.80¶
Control	8	17.1‖	214,100†§	1.26‡¶

* The labeling index data appearing here is a part of that summarized in Ref. 26. Means having the same footnote symbol differ significantly, as shown in the subsequent footnotes.
† $P < 0.01$.
‡ $P < 0.05$.
§ $P < 0.10$.
‖ $P < 0.01$.
¶ $P < 0.05$.

Continued evaluation of thymidine metabolism *in vivo* is desirable in view of the important role which this compound serves in biological investigations. In particular, the relationship between thymidine kinase levels and incorporation of [^{3}H]thymidine into DNA among different organs deserves study. Correlation coefficients of 0.84 to 0.94 have been observed for [^{3}H]thymidine in DNA versus thymidine kinase activity in regenerating rat liver, and injection of non-radioactive thymidine along with the radioactive thymidine had no significant effect on the radioactivity of the DNA over a considerable range of nmoles [30].

On summary, liver and intestinal mucosa, but not spleen or thymus of ACI strain rats incorporated significant amounts of ^{3}H from deoxyuridine[6-^{3}H] and thymidine-[methyl-^{3}H] into an RNA fraction obtained by alkaline hydrolysis of tissue homogenates. In bone marrow, ^{3}H from thymidine but not from deoxyuridine was incorporated into this fraction. Inhibition of DNA synthesis by 5-FU increased ^{3}H in this RNA fraction for only intestinal mucosa and bone marrow, while enhancement of DNA synthesis during recovery from 5-FU toxicity was associated with an increase in ^{3}H for the alkali-solubilized fractions of liver, intenstinal mucosa, spleen and thymus but not Morris hepatoma 3924A.

Acknowledgements—We thank Dr. W. B. Looney and Dr. H. P. Morris for contributions to this research. This investigation was supported by Public Health Service Research Grants CA-12758, CA-13102, CA-20516 and CA-10729 from the National Cancer Institute.

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