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Nucleotide Formation from α- and β-2′-Deoxythioguanosine in Extracts of Murine and Human Tissues[1]

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SUMMARY

Conditions for the conversion of α-2′-deoxythioguanosine (α-TGdR) and β-2′-deoxythioguanosine (β-TGdR) to nucleotides were studied in extracts of murine and human tissues. These methods were applied to assays for nucleotide formation in a series of extracts from solid tumors, neoplastic bone marrows, and normal bone marrows obtained from human patients. The results show that most of such tissue extracts can phosphorylate β-TGdR, and an appreciable percentage of extracts from the neoplasms can also phosphorylate α-TGdR. The extracts of normal bone marrows, with one exception, did not phosphorylate α-TGdR. The DNA polymerase of several tumors incorporated nucleotides of α-TGdR and β-TGdR into DNA. These results encourage the view that such tests on tumor biopsies might permit the selection of tumors with sensitivity to the deoxythioguanosines. It appears that an earlier finding in murine tissues, in which α-TGdR was converted to nucleotide and inhibited the tumors without marrow toxicity may carry over to human patients.

INTRODUCTION

A series of studies has implicated the incorporation of the antimetabolite TG[2] into DNA as the metabolic event that correlated with toxicity to the cells (1, 3, 5, 6–8). The utility of TG as a carcinostatic agent has probably been limited by the ease with which drug resistance was developed. As a means of circumventing resistance mechanisms, TGdR was tested. The β-anomer of this nucleoside was active against the cell lines resistant to TG and had toxicity comparable to that of TG on a molar basis (8). In addition, it was found that the α-anomer of the nucleoside was also active against certain TG-resistant tumors. The α-anomer was converted to nucleotide in responsive tumors but was not phosphorylated to a significant extent in the bone marrow of the mouse. As a result, the α-anomer exhibited a lower order of host toxicity in the mouse while retaining carcinostatic activity against some mouse neoplasms. In an attempt to determine whether these therapeutic advantages of α,β-TGdR would be available in the human, a test system was devised for measuring nucleotide formation in cell-free extracts prepared from murine and human tissues. The test system was applied to a variety of normal and neoplastic tissue biopsies.[3] The indications obtained in these tests encourage the view that these nucleosides may have utility in selected cancer cases.

MATERIALS AND METHODS

Thioguanine-8-^{14}C was synthesized as described earlier (3). α-2′-Deoxythioguanosine and β-2′-deoxythioguanosine were prepared, each completely free of the other, and were labeled with radiosulfur by means that have been described (4). Creatine phosphate, adenosine triphosphate, dithioerythritol, and crystalline creatine transphosphorylase were purchased from Sigma Chemical Company.

Extracts

For the study of the test conditions, Mecca lymphosarcoma ascites cells were grown in AKD2F1 female mice (AKR/J × DBA/2J) and used after 5 days of growth. Cells were allowed to swell 1 minute in H_2O at 0°C then blended for 1 minute in a Virtis 23. Solid NaCl was added to restore isotonic conditions, and blending was continued for 1 minute. This gave more activity than when the NaCl addition was omitted. The homogenates were centrifuged at 0–2°C for 1 hour at 18,000 × *g*. It was demonstrated that whole cells of Mecca lymphosarcoma could be stored at −70°C for several months without loss of activity. Extracts of murine tumor cells could be stored only a few days before activity was lost. Human tissue samples could also be stored several months without loss of activity, but extracts were, in some cases, inactive within 2 days at

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[2]Abbreviations used: TG, 6-thioguanine; α- and β-TGdR, α- and β-2′-deoxythioguanosine; TGdMP, β-2′-deoxythioguanosine-5′-monophosphate.

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−70°C. In most cases, human tissue samples were frozen as soon as obtained at surgery, stored at −70°C, and tested within a few days of collection. Human tissue samples were homogenized for 2 minutes with the Virtis 23 in isotonic saline then centrifuged 1 hour at 18,000 × *g*. The supernatant fluid was used as the crude enzyme to measure nucleotide formation. The residue was used to measure DNA as a means of quantitation of the tissue sample since this was regarded as a measure of viable tissue, and the human tissue samples were diluted with varied amounts of fat, fiber, and necrotic material. These diluents would not contain appreciable amounts of DNA.

DNA Determinations

The residue from the high-speed centrifugation was extracted twice with 0.2 M perchloric acid and twice with 95% ethanol, and the alcohol was partly removed *in vacuo*. The partially dried residue was suspended in about 10–15 volumes of 10% NaCl, the pH was adjusted to 7.5–8, and extraction of sodium nucleates was carried out by incubating for 1 hour at 100°C with intermittent stirring. The suspension was centrifuged and the supernatant fluid treated with 3 volumes of 95% ethanol in the cold. The sodium nucleates were centrifuged and the fluid discarded. The sodium nucleates were dissolved in 0.1 M NaOH and incubated 16–18 hours at 37°C to degrade RNA. Then the solution was chilled, and DNA was precipitated by adding a small excess of HCl. The DNA was dissolved in 0.1 M NaOH and read at 260 mμ in a spectrophotometer. A sample of salmon sperm DNA was used as a standard of reference.

Kinase Measurements

Nucleotide formation was measured with 1.00 ml-volumes in 13 x 100 ml pyrex tubes incubated at 37°C. Tubes were then chilled, 0.08 ml of 2.6 M perchloric acid was added to each, and the denatured proteins were centrifuged. The solutions were neutralized to pH 4.5 with 2 M KOH and kept chilled while $KClO_4$ precipitated. Then 0.100-ml aliquots were spotted on Whatman No. 1 filter paper along with "markers," and the papers were developed with 5% KH_2PO_4 by descending chromatography. The "markers" used were TG, TGdR, and TGdMP. The R_F's were approximately 0.25, 0.50, and 0.65 respectively. This chromatographic system did not separate mono-, di-, and triphosphates. The TGdMP spots were visualized under an ultraviolet lamp and cut from the papers for direct counting in a liquid scintillation system with a toluene phosphor. Efficiency was approximately 50%. The radioactivity of the substrates was determined frequently in the same manner so that corrections for decay of radiosulfur were not required.

The medium for kinase tests contained: 1.0 μmole of substrate (α-TGdR-^{35}S, β-TGdR-^{35}S, or TG-^{14}C); 10.0 μmoles creatine phosphate; 60 μg creatine transphosphorylase; 6.0 μmoles $MgCl_2$; 50 μmoles Tris buffer, pH 7.4; 1.0 μmole Na_3-ATP; 1.0 μmole dithioerythritol, and extract equivalent to 66 mg tissue. Tissue content was increased when samples appeared greatly diluted by fat or fibrosis, and usually the protein content in the assay tube would approximate 1.5–2 mg.

Since the crude extracts also contained variable amounts of phosphomonoesterases that often caused variations from a linear rate of nucleotide accumulation, three incubation times with duplicate tubes were included in each assay where the supply of extract permitted.

Polymerase

Tests on polymerase activity were made on the tissue extracts to determine whether deoxythioguanosine nucleotides were used for nucleic acid snythesis. The method was that described by York and LePage (9) with α-TGdR-^{35}S, β-TGdR-^{35}S, and 2′-deoxyguanosine-^{14}C used as test substrates. DNA was isolated as described earlier and counted in a gas-flow counter. The phosphate-generating system present in the medium (ATP + creatine phosphate + creatine transphosphorylase) was that used in the assays for nucleotide formation. It has been demonstrated (4) that nucleoside mono-, di-, and triphosphates of α-TGdR and β-TGdR were all formed in murine tumor cells. The triphosphate would be the necessary substrate for the polymerase activity. The isobutyric acid-ammonia-water system of paper chromatography used earlier (4) was used for the present study in two instances to analyze the nucleotides formed by human tumor extracts assayed for polymerase activity.

RESULTS AND DISCUSSION

Extracts of the Mecca lymphosarcoma, a murine tissue, were used as a model system to determine the conditions required for the assay of capacity to form nucleotides from these α- and β-nucleoside analogs. Some indications were provided that the same conditions would be suitable for the assays with human tissues. However, each human tissue could be expected to vary in the balance of enzymes present in this crude system. For example, they would vary in the balance between kinases and phosphatases. The data presented in Table 1 indicate that the pH used has little effect over a relatively broad range, with some indication of an optimum at pH 7.4. A phosphate bond energy-generating system, ATP and magnesium ion, were necessary. Some reduction in creatine phosphate level was tolerated for shorter incubation times. The rates were linear for 10 or 20 minutes. Addition of potassium ion was unnecessary, possibly because endogenous potassium levels were sufficient. Dithioerthyritol appeared unnecessary in the Mecca lymphosarcoma extracts, but its use has been continued because of a demonstrated ability to stabilize enzymes under marginal conditions. The nucleotide formation was proportional to the amount of tissue extract added. Substrate levels were adequate for the amounts of extract being used. Data for 5-, 10-, and 20-minute incubations were included for 2 human tumor samples as examples to illustrate that phosphorylation did show a lag at the earliest time in rare instances and that it was not unusual to have a reduced rate by 20 minutes, presumably because of phosphatase activity that began to outpace the nucleotide synthesis.

Some data are presented in Table 2 to show how assays varied in tests with different portions of the same tumor. While there were appreciable variations, the conclusions as to whether a tumor was relatively active or relatively inactive in

Table 1

Tissue extract	Substrate	Incubation time (min)	Variation	Nucleotide formed (mμmoles/gm)
Mecca lymphosarcoma	β-TGdR-^{35}S	5, 10	pH 6.8	33, 57
Mecca lymphosarcoma	β-TGdR-^{35}S	5, 10	pH 7.1	30, 62
Mecca lymphosarcoma	β-TGdR-^{35}S	5, 10	pH 7.4	38, 70
Mecca lymphosarcoma	β-TGdR-^{35}S	5, 10	pH 7.7	33, 67
Mecca lymphosarcoma	β-TGdR-^{35}S	5, 10	pH 8.1	26, 51
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20		62, 128
Mecca lymphosarcoma	β-TGdR-^{35}S	10	−MgCl	11
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20	−ATP	21, 19
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20	5 μmoles creatine phosphate (instead of 10 μmoles)	62, 104
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20	− Dithioeryritol	74, 124
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20	+ 6 μmoles KCl	56, 119
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20	2.0 μmoles substrate	61, 129
Mecca lymphosarcoma	β-TGdR-^{35}S	10, 20	Doubled enzyme level	120, 242
Mecca lymphosarcoma	TG-^{14}C	10, 20		2, 1
Mecca lymphosarcoma	α-TGdR-^{35}S	5, 10, 20		19, 37, 75
Mecca lymphosarcoma	β-TGdR-^{35}S	5, 10, 20		35, 68, 47
Human marrow #1	α-TGdR-^{35}S	5, 10, 20	pH 7.4	0, 0, 0
Human marrow #1	β-TGdR-^{35}S	5, 10, 20	pH 7.4	22, 54, 110
Human marrow #2	β-TGdR-^{35}S	5, 10	pH 7.1	38, 83
Human marrow #2	β-TGdR-^{35}S	5, 10, 20	pH 7.4	48, 102, 200
Human marrow #2	β-TGdR-^{35}S	5, 10	pH 7.7	42, 80
Human marrow #2	α-TGdR-^{35}S	5, 10	pH 7.1	15, 42
Human marrow #2	α-TGdR-^{35}S	5, 10, 20	pH 7.4	6, 27, 60
Human marrow #2	α-TGdR-^{35}S	5, 10	pH 7.7	23, 55
Human carcinoma of lung (G. S.)	α-TGdR-^{35}S	5, 10, 20		17.5, 32.9, 46.0
Human carcinoma of lung (G. S.)	β-TGdR-^{35}S	5, 10, 20		17.9, 42.8, 76.0
Human adenocarcinoma of ovary (V. F.)	α-TGdR-^{35}S	5, 10, 20		6.8, 14.0, 21.3
Human adenocarcinoma of ovary (V. F.)	β-TGdR-^{35}S	5, 10, 20		49.8, 109, 150

Conditions required for nucleotide formation. Abbreviations: α,β-TGdR-^{35}S, α,β-2′-deoxythioguanosine-6-^{35}S. Results are expressed per gm wet weight of tissue equivalent. The Mecca lymphosarcoma experiments were performed with fresh tissue extract and were repeated on a part of the same extract frozen and stored at −70°C for 7 days. The results for the 2 experiments were almost identical and were average (i.e., duplicate experiments and duplicate incubations were averaged). The other results are for averages of duplicate tubes in single experiments. Unincubated samples were used as a check on possible radiochemical contamination of the product area by substrate impurities. This involved a small correction. Analyses showed: Mecca lymphosarcoma, 3.05 mg DNA/gm; human marrow #1 was obtained at autopsy from an elderly female, 1.0 mg DNA/gm; human marrow #2 was obtained from a child with an infection, 1.01 mg DNA/gm; human carcinoma of lung, 3.20 mg DNA/gm; human adenocarcinoma of ovary, 0.10 mg DNA/gm. The marrows were obviously diluted with blood.

forming nucleotide from either α-TGdR or β-TGdR, or both, probably would not be affected by such variations as were found.

Table 3 presents assays on a range of human tissue samples, including solid tumors and peripheral white cells from leukemic patients and bone marrows. Some of the marrow samples, as indicated by the diagnosis, would be mainly neoplastic cells. Others, such as those from patients with lung carcinomas, should be relatively normal.

An initial assumption that analysis for DNA would provide a measure of active tissue appears to be generally valid. The low DNA values seen in some instances, such as in certain carcinomas of the breast, seemed to reflect high fat content. However, some samples, such as the carcinoma of the pancreas (M. V.) noted, appear to show very low DNA values because they rapidly degraded the DNA before they were frozen. In this particular example, freezing after surgical removal was very prompt, but nuclease activity was apparently exceptionally

Table 2

Tissue	DNA content (mg/gm)	Substrate	Nucleotide formed (mμmoles/mg DNA) 5 min	10 min	20 min
Sarcoma of lumbar region (E. M.)	0.778	α-TGdR-^{35}S	0	0	0
Sarcoma of lumbar region (E. M.)		β-TGdR-^{35}S	30	45	57
Sarcoma of lumbar region (E. M.)	0.306	α-TGdR-^{35}S	0	0	0
Sarcoma of lumbar region (E. M.)		β-TGdR-^{35}S	53	95	117
Carcinoma of breast (E. S.)	0.049	α-TGdR-^{35}S		156	78
Carcinoma of breast (E. S.)		β-TGdR-^{35}S		60	141
Carcinoma of breast (E. S.)	0.149	α-TGdR-^{35}S	44	59	95
Carcinoma of breast (E. S.)		β-TGdR-^{35}S	40	55	123
Carcinoma of lung (W. T.)	0.679	α-TGdR-^{35}S	48	86	120
Carcinoma of lung (W. T.)		β-TGdR-^{35}S	45	77	111
Carcinoma of lung (W. T.)	0.433	α-TGdR-^{35}S	57	106	142
Carcinoma of lung (A. T.)	0.255	α-TGdR-^{35}S	169	202	299
Carcinoma of lung (A. T.)		β-TGdR-^{35}S	195	175	112
Carcinoma of lung (A. T.)	0.192	α-TGdR-^{35}S	135	260	158
Carcinoma of lung (A. T.)		β-TGdR-^{35}S	148	153	219

Analyses of replicate samples from tumors. Tumors of sufficient size were cleaved into 2 fragments while frozen, and the 2 portions were analyzed as if they were separate tumors to check the effect that tumor heterogenicity would have on the conclusions. Each figure is an average from duplicate incubations. Abbreviations are as in Table 1.

high. It would thus appear desirable to have some assessment of the condition of the tumor by pathologists as a supplement for the data on DNA content or wet weight.

The white blood cell and marrow samples were obtained aseptically. However, there is a potential for microbial contamination in solid tumor samples. This would be minimized or eliminated by the procedures used. Necrotic areas were avoided. Microbial cells require relatively drastic procedures for cell lysis (2), and whole cells would be removed by the high-speed centrifugation used in the preparation of extracts for the enzyme assays.

Table 4 presents data on a series of human tumor samples, extracts of which were tested for ability to incorporate α- and β-TGdR into DNA. It has been established that, in crude extracts of murine tissues, there is little polymerase activity if any of the components are omitted (9); i.e., denatured DNA, triphosphates of all four nucleotides, and a phosphorylative support system were all necessary. These omissions from the medium were not routinely tested in the assays presented in Table 4 for human tissues. However, the analyses were routinely corrected for small amounts of radioactivity present in unincubated samples. In two instances tested, the omission of accessory components led to loss of the observed activity. In two instances, the acid-soluble extracts were later analyzed by paper chromatography in isobutyric acid-ammonia-water after 0- and 15-minute incubations. The extracts from the 15-minute incubations were found to contain nucleotides, as indicated in Table 5. With one exception, where the extract was also unable to make nucleotides, the extracts all incorporated β-TGdR into DNA. Several of the samples also incorporated α-TGdR into DNA. One sample of uninvolved liver showed very low activity, in line with the low mitotic index for this tissue. These results would encourage the conclusion that measurements of nucleotide formation would predict whether the nucleoside would be incorporated into DNA. The normal DNA precursor, deoxyguanosine, was poorly utilized. This results largely from the rapid degradation of deoxyguanosine in such tissue extracts.

The results presented herein would encourage the view that the low toxicity of α-TGdR in the mouse, reported earlier (8) to be due to lack of phosphorylation in the marrow, would probably apply in humans; it appeared that an appreciable fraction of human tumors was able to phosphorylate this analog and might therefore be responsive to treatment with α-TGdR. In those tumors where α-TGdR was not converted to nucleotide at appreciable rates, the formation of nucleotide from β-TGdR would have significance. As discussed earlier (8), β-TGdR would have advantages in such tumors over TG because of the potential for development of drug resistance that is a limitation of TG. Four different mechanisms have been found by which various mouse tumor cell lines achieve resistance to TG (8). β-TGdR, in a tissue where it can be phosphorylated to nucleotide, represents a means of by-passing all four of these mechanisms. In tumors that phosphorylate α-TGdR, this same advantage would be achieved without the price of toxicity to bone marrow elements. Actual determination as to whether these objectives can be achieved obviously must await clinical trials with α- and β-TGdR.

Table 3

Tissue	DNA content (mg/gm)	Nucleotide formed (mμmoles/mg DNA/hr) α-TGdR-^{35}S	β-TGdR-^{35}S
Esophageal carcinoma (2/14/67)	2.44	0	252
Carcinoma of pancreas (M. V.)	0.09[a]	745	0
Carcinoma of breast (G. D.)	1.64	38	95
Carcinoma of lung (A. T.)	0.255	2,040	2,345
Carcinoma of lung (W. T.)	0.679	578	542
Carcinoma of breast (E. S.)	0.149	523	483
Sarcoma of lumbar region (E. M.)	0.778	0	362
Squamous Cell Carcinoma of the back (179-15194)	0.184	640	1,130
Carcinoma of colon (M. S.)	0.016[a]	10,400	4,870
Carcinoma of breast (D. M.)	0.017[a]	7,700	16,800
Carcinoma of small bowel (P. L.)	0.072	5,150	5,700
Adenocarcinoma of testicle (C. W.)	0.999	263	620
Malignant spleen of 7-year male (L. W.)	0.030[a]	7,170	5,330
Carcinoma of breast (Jones)	0.248[b]	278	504
Adenocarcinoma of ovary (F. V.)	0.095	884	6,910
Carcinoma of breast (Mulligen)	1.34	209	369
Carcinoma of lung (G. S.)	3.20	66	80
Carcinoma of breast (S. B.)	3.11	23	181
Squamous cell carcinoma of leg (J. H.)	0.633	174	353
Carcinoma of breast (G. C.)	1.65	78	379
Carcinoma of colon (F. J.)	0.22	527	1,790
Carcinoma of breast (M. L.)	0.43	0	482
Bone marrow, Hodgkins untreated (Baca)	1.02	8,100	292
Bone marrow, carcinoma of lung treated with PDA (Solomonson)	1.875	0	0
Bone marrow, carcinoma of lung treated with cytosine arabinoside (Rivers)	0.400	0	350
Bone marrow, myeloma untreated (Holiock)	1.48	0	0
Bone marrow, myeloma untreated (Hulett)	1.50	0	0
Bone marrow, lymphoma untreated (Walker)	0.183	0	0
Bone marrow, myeloma untreated (Irving)	0.950	157	893
Bone marrow, acute myeloid leukemia treated with MTX (Martineau)	1.98	32	1,980
Bone marrow, carcinoma of lung untreated (W. Hawkins)	0.16	0	5,000
Bone marrow, myeloma treated with myleran (C. Villa)	0.120	1,800	166
Bone marrow, myeloma treated with hydroxyurea (L. Shinn)	0.234	690	1,620
Bone marrow, chronic granulocytic leukemia treated with 6-MP (Woolfort)	1.185	21	1,920
White blood cells, chronic lymphocytic leukemia untreated 4/6/67 (Butt)	6.67	24	106
White blood cells, CLL treated with prednisone 5/18/67 (Butt)	4.72	2	316
White blood cells, CLL untreated 9/14/67 (Butt)	4.75	58	135
White blood cells, CLL untreated 10/12/67 (Butt)	1.05	1,230	5,640
White blood cells, acute granulocytic leukemia (Stout)	5.6	59	1,430
White blood cells, lymphoblastic lymphosarcoma untreated (M. Anderson)	2.06	32	120
White blood cells, lymphoblastic lymphosarcoma treated with Vincristine (C. Dodds)	3.67	0	229
White blood cells, carcinoma of neck region (Bailey)	3.08	16	115
White blood cells, lymphoma, untreated (Baker)	0.625	38	107
White blood cells, lymph node (Baker)	1.61	0	10
White blood cells, acute leukemia untreated (Blanchard)	4.26	515	199
White blood cells, acute leukemia untreated (S. Tong)	4.25	102	335

Table 3 (Continued)

Tissue	DNA content (mg/gm)	Nucleotide formed (mμmoles/mg DNA/hr) α-TGdR-^{35}S	β-TGdR-^{35}S
White blood cells, chronic granulocytic leukemia treated with myleran (L. McCarter)	3.86	0	436
White blood cells, lymphoma untreated (R. Braly)	1.60	74	250
White blood cells, chronic granulocytic leukemia treated with TMCA (C. Lowe)	3.60	1,060	805
White blood cells, lymphosarcoma treated with TMCA (Crostley)	2.43	4,470	3,890
White blood cells, acute myeloblastic leukemia untreated (Durka)	2.49	824	475
White blood cells, chronic granulocytic leukemia treated with 6-MP (Woolford)	5.5	138	198
Carcinoma of breast (E. F.)	0.020	350	8,250
Sarcoma of abdominal wall, recurrent (F. S.)	0.100	1,530	2,930
Liposarcoma of leg (D. G.)	0.133	1,640	1,100
Carcinoma of colon (C. O.)	0.110	2,690	3,570
Carcinoma of breast (B. R.)	0.384	690	1,030
Carcinoma of breast (M. A.)[b]	0.012	1,200	1,375
Carcinoma of breast (G. W.)	1.11	111	835
Carcinoma of breast (G. A.)	0.425	0	0
Carcinoma of breast (A. H. 68-41-52)	0.400	0	2,700
Carcinoma of breast (S. K.)	0.243	1,000	5,150
Tumor of spleen (O. B.)[a]	0.024	2,900	19,000
Tumor of leg muscle (G. K. 68-1324)	0.163	1,230	3,080
Tumor of testicle (R. E. 281-38-47)	0.300	297	3,750
Carcinoma of stomach (J. O. H.)	0.035[a]	5,340	41,300
Carcinoma of breast (B. E. V. 283-40-35)	[c]	807	1,940
Carcinoma of breast (D. E.)	0.153	2,110	1,170
Sarcoma of gluteal muscle (P. C.)	0.244	0	0
Carcinoma of breast (B. B.)	0.108	3,460	3,600
Adenocarcinoma of testicle (S. S.)	0.073	2,430	19,540
Tumor in muscle of hand (F. U.)	0.180	1,250	1,200
Sarcoma of leg muscle (A. M. 68-4294)	0.278	400	939
Ductal Carcinoma (C. M. 282-76-45)	0.055	1,602	0

Rate of nucleotide formation by extracts of human tissues *in vitro*. Solid tumor samples were frozen, stored at −70°C, and assayed within a few days. White blood cell samples were separated by settling at 0–2°C and drawing off the "buffy coat," then centrifuging the cells. They were essentially free of erythrocytes. In both cases, samples were blended 2 minutes at 23,000 rpm in a Virtis 23 at 0°C and centrifuged at 18,000 × *g* for 1 hour at 0°C. The supernatant fluid was used for the enzyme assays and the pellet for DNA measurements. Duplicate incubations were carried out for 5, 10, and 20 minutes. The figures represent maximum rates of nucleotide formation; in almost all cases this occurred at 5 or 10 minutes of incubation. Incubations with thioguanine-8-C^{14} were included in many of the earlier analyses but gave negative results. This indicated that the conditions did not permit cleavage and recycling of thioguanine to ribonucleotides. Abbreviations: PDA, phosphodiaminic acid; α-, β-TGdR-^{35}S, α,β-2′-deoxythioguanosine-6-^{35}S; TMCA, trimethylcholchicinic acid; MTX, methotrexate; 6-MP, 6-mercaptopurine; CLL, chronic lymphocytic leukemia.

[a] Samples that appeared cellular. Low DNA values probably resulted from high nuclease activity.

[b] Sample with very high fat content as a diluent.

[c] Per gram wet weight. DNA analysis lost.

Table 4

Tissue	DNA content (mg/gm)	Incorporation into DNA (mμmoles/mg DNA/hr) α-TGdR-^{35}S	β-TGdR-^{35}S	GdR-^{14}C
Testicular tumor (Rauteria)	1.84	98	21,300	1.1
Sarcoma of abdominal wall (Solana)	2.96	69	393	2.0
Squamous cell carcinoma of upper leg (Hurley)	1.14[a]	0	0	0
Hepatoma (P. U.)	1.46	0	21,700	0.4
Uninvolved liver (P. U.)	1.33	0	38	3.2
Carcinoma of stomach (L. J.)	0.56[b]	157	543	
Carcinoma of breast (D. M.)	0.153[b]	1630	830	
Liposarcoma of leg muscle (G. O.)	0.805	144	54	
Carcinoma of breast (G. G.)	1.04	355	340	

Polymerase activity of extracts from human tissue. Each tube contained 15 mμmoles of thymidine triphosphate; 15 mμmoles of deoxycytidine triphosphate; 15 mμmoles of deoxyadenosine triphosphate; 50 μmoles of Tris-HCl buffer, pH 7.9; 6 μmoles of $MgCl_2$; 10 μmoles of creatine phosphate; 60 μg of creatine transphosphorylase; 1 μmole of ATP; 100 μg of denatured DNA; cell-free extract of the tumor equivalent to 65 mg of tissue; and 1.0 mμmole of substrate (α-TGdR-^{35}S, α-2′-deoxythioguanosine-6-^{35}S; β-TGdR-^{35}S, β-2′-deoxythioguanosine-6-^{35}S; or GdR-^{14}C, β-2′-deoxyguanosine-8-^{14}C); all was in a volume of 0.70 ml.

[a] No mononucleotide formation; probably a deteriorated sample.

[b] Sample with very high fat content.

Table 5

Tissue	Substrate	Nucleotide found (mμmoles/mg DNA) Mono-	Di-	Triphosphate
Testicular tumor (Rauteria)	α-TGdR-^{35}S	564	0	13
	β-TGdR-^{35}S	47	87	17
	TG-^{14}C	6	9	7
Hepatoma (P. U.)	α-TGdR-^{35}S	0	504	55
	β-TGdR-^{35}S	0	20	62
	TG-^{14}C	0	0	0

Analysis of nucleotides formed from deoxythioguanosines by extracts of human tissues. Analyses are averages from duplicate samples incubated 15 min, deproteinized with perchloric acid, neutralized to pH 4.5 with KOH, and chromatographed on Whatman 3MM papers with isobutyric acid-ammonia-water (4) with thioguanosine-5′-mono-, -di-, and -triphosphate (TGMP, TGDP, and TGTP) as carriers. The spots were visualized with an ultraviolet lamp, cut out and counted in a toluene scintillator. The analyses have been corrected for a small amount of radioactivity present in spots from unincubated samples but do not include nucleotides present in the DNA obtained from the incubations. See Footnote 1 for other abbreviations.

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