lar compounds has been previously described by others.[1]

TABLE I

CARCINOSTATIC ACTIVITY OF SOME 2-AMINO-1,3,4-THIADIAZOLES

R	S91 Melanoma Daily dose, mg./kg.	S91 Melanoma % Tumor[a] inhibition	8110 Glioblastoma Daily dose, mg./kg.	8110 Glioblastoma % Tumor[a] inhibition	6C3HED Lymphosarcoma Daily dose, mg./kg.	6C3HED Lymphosarcoma % Tumor[a] inhibition
2-R-Amino-1,3,4-thiadiazole						
H	25	94	120	71	100	99
	3.75	57	100	51	50	94
CH_3	250	94	500	61		
	187	78	375	40		
C_2H_5	100	88	200	63	250	94
	15	70	150	51		
Allyl	125	89	125	62	100	28
	62.5	82	62.5	26		
Phenyl	150	0	37.5	8		
	75	0	12.5	0		
Acetyl	100	81	200	78	150	61
	75	33	100	23	75	52
2-Amino-5-R-1,3,4-thiadiazole						
OH	187	54	500	70		
			250	50		
SH	500	27	500	0	500	0
	250	0	250	0	250	0
Cl	100	87	100	10	250	87
	75	76	75	31	125	80
2-R-Amino-5-methyl-1,3,4-thiadiazole						
CH_3	300	20	300	0		
	125	0	125	15		
Allyl	250	30	250	0		
	125	0	125	0		

[a] % tumor inhibition = $100 - \frac{\text{Av. tumor weight of treated mice} \times 100}{\text{Av. tumor weight of control mice}}$

The tumors used were the S-91 melanoma of the DBA-line 1 mouse, the 8110 glioblastoma of the A mouse and the 6C3HED lymphosarcoma of the C3H mouse. These tumors were implanted into the appropriate strain of mouse and allowed to become established before treatment was started. The compounds were given in daily intraperitoneal doses at the levels indicated in Table I. The melanoma was treated for two weeks, the other tumors for one week. The tumors were then excised and weighed. The highest doses shown are approximately the maximum tolerated doses of the compounds.

From the results shown in Table I the parent compound, 2-amino-1,3,4-thiadiazole, appears to be the most active. The 2-lower alkylamino and 2-acylamino derivatives were also active and less toxic than the parent amino compound, while the 2-phenylamino derivative was inactive. In most cases substitution in the 5 position reduced the activity of the 2-amino derivatives.

Acknowledgment.—We wish to acknowledge the helpful advice of Dr. Sidney Farber in evaluating these experiments.

J. J. OLESON
A. SLOBODA
W. P. TROY
AMERICAN CYANAMID COMPANY
S. L. HALLIDAY
RESEARCH DIVISION
M. J. LANDES
LEDERLE LABORATORIES
R. B. ANGIER
PEARL RIVER, NEW YORK
J. SEMB
K. CYR
J. H. WILLIAMS

RECEIVED OCTOBER 21, 1955

(1) (a) L. L. Bambas, "The Chemistry of Heterocyclic Compounds," Interscience Publishers, Inc., New York, N. Y., 1952; (b) M. Freund and H. P. Schwartz, *Ber.*, **29**, 2487 (1896).

THE OCCURRENCE OF DEOXY-PYRIMIDINE NUCLEOTIDES IN THE ACID-SOLUBLE EXTRACT OF THYMUS[1]

Sir:

A previous report suggesting the natural occurrence of thymidinetriphosphate and possibly other deoxy-nucleotides has appeared.[2] The recently reported[3] synthesis *in vitro* of ribo-polynucleotides from diphospho-ribonucleotides with a soluble enzyme preparation from *Azotobacter vinelandii* has drawn attention to the high-energy nucleotides as direct precursors of polynucleotides. Although Kanazir[4] has reported finding thymidylic acid in soluble extracts of *E. coli* and Schneider[5] has reported finding deoxy-pyrimidine nucleosides in rat tissue extracts, no one, to the authors' knowledge, has reported finding deoxy-nucleotides in soluble extracts of mammalian tissues. This report presents evidence for the occurrence of the mono-, di- and triphosphate derivatives of thymidine and deoxycytidine in cold perchloric acid extracts of fresh calf thymus.

The neutralized extract was chromatographed on Dowex-1 by extended gradient elution[6] with the formic acid system. A compound tentatively identified as TTP,[7] but poorly resolved from GTP and UTP, was hydrolyzed in *N* HCl and rechromatographed in the AM-F system[6] to yield TMP. Analytical data, in μM./μM. of nucleotide (amounts based on ultraviolet spectral data), were: deoxyribose,[8] 0.93; 5′-P,[6] 0.96; and total P, 0.99. On paper chromatography in three-solvent systems, the sample exhibited essentially the same R_f's as authentic TMP (Table I). Hydrolysis of this TMP at the glycosidic bond gave a compound which had an R_f identical to thymine in three solvent systems (Table II). Incomplete enzymatic hydrolysis of the TTP peak by potato apyrase[9] gave TMP and

(1) This work performed under Atomic Energy Commission Contract No. AT(11-1)-75.

(2) R. L. Potter, *Fed. Proc.*, **14**, 263 (1955).

(3) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955); M. Grunberg-Manago and S. Ochoa, Abstracts of Papers Presented at the American Chemical Society Meetings, Sept. 11–16, 1955.

(4) D. Kanazir, *Biochim. et Biophys. Acta*, **13**, 589 (1954).

(5) W. C. Schneider, *J. Biol. Chem.*, **216**, 287 (1955).

(6) R. B. Hurlbert, H. Schmitz, A. Brumm and V. R. Potter, *ibid.*, **209**, 23 (1954).

(7) The following abbreviations have been used: R_f, ratio of the movement of a band to the movement of the solvent front; AM-F, ammonium formate; TMP, TDP, TTP, the mono-, di-, and triphosphates of thymidine; ATP, adenosine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate; CMP, CDP, CTP, D-CMP, D-CDP, D-CTP, the mono-, di-, and triphosphates of cytidine and deoxy-cytidine, respectively.

(8) S. Brody, *Acta Chem. Scand.*, **7**, 502 (1953).

(9) P. S. Kirshnan, *Arch. Biochem.*, **20**, 261 (1949).

TABLE I

PAPER CHROMATOGRAPHY OF THYMIDYLIC ACID OBTAINED BY ACID HYDROLYSIS OF THE THYMIDINETRIPHOSPHATE PEAK[a]

System	Rf Sample	Rf TMP
Isopropyl alcohol–HCl[b]	0.91	0.91
Isobutyric acid–ammonia[c]	.59	.59
N-Propyl alcohol–ammonia[d]	.26	.29

[a] Hydrolysis, 10 minutes in *N* HCl at 100°. [b] G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951). [c] See reference (9). [d] C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

TDP which were separated by ion-exchange chromatography.

TABLE II

PAPER CHROMATOGRAPHY OF A BASE FROM THE THYMIDINETRIPHOSPHATE PEAK[a]

System	Rf Sample	Rf Thymine
Isopropyl alcohol–HCl[b]	0.79	0.79
Butyl alcohol–water[c]	.52	.52
Isobutyric acid–ammonia[d]	.81	.81

[a] An aliquot of the TMP sample obtained by acid hydrolysis of the TTP peak (*cf.* Table I) was hydrolyzed 1 hour in 98% formic acid at 175° in a sealed tube. [b] See footnote (*b*) Table I. [c] R. Markham and J. D. Smith, *Biochem. J.*, **45**, 294 (1949). [d] See reference (9).

TTP obtained from another chromatogram was purified by paper chromatography.[9] The isolated material with a D_{275}/D_{260} ratio of 0.92 contained per μM. of TTP: deoxyribose, 0.75 μM.; acid-labile P, 1.55 μM.; and total P, 2.47 μM. Deoxyribose: acid-labile P: total P = 0.92:1.88:3.00.

The only evidence for TDP *per se* was the appearance of 2.0 μM. of TMP in an enzymatic (apyrase) hydrolysate of the ATP peak. Chromatographic evidence[11] and technical considerations[6,12] suggest that TDP, if it exists, would appear in or near the ATP peak.

Thymidylic acid (2.0 μM.) was found by rechromatography of the UMP peak (AM-F system): deoxyribose, 1.02 μM./μM. of TMP and total P, 0.97 μM./μM. of TMP.

Acid hydrolysis of the CTP peak gave a mixture of cytidylic acids which could be resolved into CMP and D-CMP by either ion-exchange using the borate technique[13] or paper chromatography.[10] In addition, paper chromatography[9] of the CTP peak purified on the AM-F system[6] gave only two bands, each of which had identical spectra in acid (maximum at 280 mμ, minimum at 242 mμ) and alkali. The faster moving band contained per μM. of D-CTP: deoxyribose, 0.86 μM.; acid-labile P, 1.75 μM.; and total P, 2.62 μM.

Rechromatography of the CDP peak in the AM–F system gave what appeared to be only CDP 10.4 μM.). A Brody test, however, indicated 0.41 μM. of deoxyribose/μM. of nucleotide. Paper chromatography[9] resolved the mixture into CDP and D-CDP. The latter contained: deoxyribose, 0.87 μM.; acid-labile P, 0.87 μM.; and total P, 1.8 μM. (data expressed per μM. of D-CDP).

By the same technique the CMP peak yielded about one μM. of D-CMP.

It should be pointed out that all of the cytidine nucleotide preparations so far examined contained deoxyribose and ribose in roughly a 40:60 ratio.

No evidence for deoxypurine nucleotides has been found.

ATOMIC ENERGY COMMISSION
BIOLOGICAL EFFECTS OF IRRADIATION LABORATORY
UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN
RICHARD L. POTTER
SONDRA SCHLESINGER

RECEIVED NOVEMBER 1, 1955

(10) H. A. Krebs and R. Hems, *Biochim. et Biophys. Acta*, **12**, 172 (1953).

(11) Data to be presented in a more complete publication.

(12) L. I. Hecht, V. R. Potter and E. Herbert, *Biochim. et Biophys. Acta*, **15**, 134 (1954).

(13) J. X. Khym and W. E. Cohn, *ibid.*, **15**, 139 (1954).

CO-FACTOR REQUIREMENTS FOR THE INCORPORATION OF $H_2C^{14}O$ AND SERINE-3-C^{14} INTO METHIONINE[1]

Sir:

Using $H_2C^{14}O$ and serine-3-C^{14} as one-carbon sources, we have investigated the co-factor requirements for the *in vitro* incorporation of these compounds into methionine methyl groups by cell-free extracts of sheep liver. The methyl groups of methionine were assayed by cleavage with HI[2] and trapping the evolved CH_3I with trimethylamine.[3] The requirements for Mg^{++}, ATP, DPN and homocysteine have been shown with either H_2CO or serine as the one-carbon source, whereas, the co-factor effect of leucovorin is apparent only when H_2CO is utilized (Table I). The requirement for pyridoxal phosphate (PLP), although not apparent from the data of Table I, is demonstrated in Table II where data for the pre-incubation of enzyme with PLP, ATP, and deoxypyridoxine (DPX) in various combinations indicate that the inhibition of incorporation of both $H_2C^{14}O$ and serine-3-C^{14} by DPX can be partially reversed by PLP.

TABLE I

CO-FACTOR REQUIREMENTS FOR THE INCORPORATION OF SERINE-3-C^{14} AND $H_2C^{14}O$ INTO METHIONINE

Each vessel contained 20 μmoles DL-homocysteine, 20 μmoles DL-serine-3-C^{14} (14,100 ct./min./μmole) or 11.4 μmoles $H_2C^{14}O$ (16,800 ct./min./μmole), 1 μmole L-methionine, 10 μmoles $MgSO_4$, 20 μmoles ATP, 2 μmoles DPN, 1 μmole pyridoxal phosphate, 400 γ leucovorin, 200 μmoles tris-(hydroxymethyl) aminomethane (Tris) at *p*H 7.3, and 1 ml. enzyme. Enzyme was prepared by homogenizing 1 part sheep liver with 2 parts Tris buffer, 0.1 *M*, *p*H 7.3, and dialyzing the supernatant fluid obtained by centrifuging at 100,000 × *g* against 0.025*M* Tris buffer for 24 hours. Final vol. 3 ml., incubated under nitrogen at 37° for 2 hours.

Omission	Counts in methionine methyl groups: Serine-3-C^{14} ct./min.	Counts in methionine methyl groups: $H_2C^{14}O$ ct./min.
None	15,650	3800
Homocysteine	220	380
Methionine	3,660	780
$MgSO_4$	320	85
ATP	55	0
DPN	4,430	925
Pyridoxal phosphate	14,400	4210
Leucovorin	13,900	1070

(1) Aided by research grants from the National Institutes of Health, U. S. Public Health Service, and the California Division of the American Cancer Society.

(2) H. D. Baernstein, *J. Biol. Chem.*, **106**, 451 (1934); **115**, 25 (1936).

(3) W. Sakami, *ibid.*, **187**, 369 (1950).