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[24] The following abbreviations are used: αC-RNA, complementary, symmetric RNA whose enzymatic synthesis has been catalyzed by purified RNA polymerase; αRNA, RNA whose synthesis on an αDNA template has been catalyzed by crude extracts; α_LDNA, the pyrimidine-rich and more buoyant isolated "strand" of αDNA; Tris, tris (hydroxymethyl) aminomethanol; 2 SSC, 0.3 *M* NaCl, 0.03 *M* sodium citrate, pH 7; A, G, C, and U, adenine, guanine, cytosine, and uracil; GRP^{32}PP, guanosine 5′ triphosphate labeled in the ribose proximal phosphate with P^{32}; CTP, cytidine 5′ triphosphate.

BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF THE ANALOGUE ANTIBIOTIC TUBERCIDIN

BY G. ACS, E. REICH, AND M. MORI

INSTITUTE FOR MUSCLE DISEASE, AND THE ROCKEFELLER INSTITUTE, NEW YORK

Communicated by E. L. Tatum, June 24, 1964

Tubercidin (7-deazaadenosine; 4-amino pyrrolo (2,3-d) pyrimidine β-D-ribofuranoside), an analogue of adenosine (Fig. 1), is found in culture filtrates of *Streptomyces tubercidicus*.[1] The structure of tubercidin (Tu) is well established,[2] and the chemical synthesis of a related derivative has been achieved.[3] It has been reported that tubercidin inhibits the growth of some experimental tumors,[4, 5] but its toxicity to mice is rather low.[4, 5] In this paper we describe the metabolism of Tu in mouse fibroblasts (strain L-929) and some effects of this antibiotic on cellular and viral functions. The results show that tubercidin is incorporated into both cellular nucleic acids. The consequences of this incorporation, which occurs even when Tu is present at very low concentrations in the culture media, are lethal both for mouse fibroblasts and for some viruses which are capable of growing in these cells.

Materials and Methods.—(1) *Cells and viruses:* Mouse fibroblasts were maintained and propagated either as monolayer cultures as previously described,[6] or in suspension cultures. The medium was minimal Eagle's medium (MEM)[7] supplemented with fetal bovine serum (5% for monolayers, 10–20% for suspension cultures). Vaccinia virus, Mengovirus, and Reovirus were grown and assayed, and the growth of monolayer cultures observed as previously reported.[6, 8, 9]

(2) *(4-Amino pyrrolo (2,3-d) pyrimidine)* was the gift of Dr. G. Hitchings, The Wellcome Research Laboratory, Tuckahoe, N. Y. Dr. C. G. Smith of the Upjohn Co., Kalamazoo, Mich., generously provided tubercidin. On paper chromatography this product showed a single UV-absorbing spot whose spectrum corresponded to that reported for tubercidin;[2] in some batches a small amount of contaminating fluorescent material was noted.

(3) *Radioactive H^3-tubercidin:* Authentic pure Tu was tritiated (Nuclear-Chicago Corp.) by exchange with H^3 in glacial acetic acid. The crude radioactive product was purified to a single, chromatographically homogeneous material of constant specific radioactivity (1.75×10^6 cpm μg) by paper chromatography in isobutyric acid—0.5 *N* NH_4OH (5:3), and by paper electrophoresis (0.04 *M* citrate buffer pH 3.5).

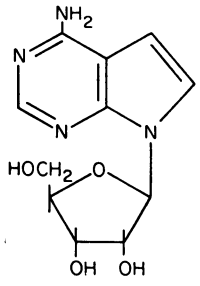

FIG. 1.—Structure of tubercidin. The N-7 of adenine is replaced by C-H.

(4) *Deaminated tubercidin (7-deazainosine; 4-hydroxy-pyrrolo(2,3-d)-pyrimidine ribofuranoside; desaminotubercidin):* 1 mg radioactive tubercidin was incubated at 20°C in 0.2 *M* acetate buffer pH 4.2, containing sodium nitrite (1 *M*). The progressive deamination of Tu was followed by paper electrophoresis of aliquots of the reaction mixture at timed intervals. When the reaction was complete (24 hr), the product was purified by adsorption and elution from charcoal and by paper electrophoresis (pH 3.5, 0.05 *M* citrate buffer). The spectrum of this product was identical with that reported recently[3] for synthetic deaminated Tu.

(5) *Deoxytubercidin (7-deaza-2'-deoxyadenosine):* L-cells (1.2×10^6/ml) were incubated for 16 hr in MEM containing 1 μg/ml radioactive H^3-tubercidin. The cells were harvested by centrifugation, washed twice with saline, three times with perchloric acid, once each with ethanol-ether (3:1) and ether. Following incubation in 0.5 *N* KOH (37°, 18 hr), the residue which precipitated on acidification was washed with trichloroacetic acid, ethanol-ether, and ether, and incubated (37°, 6 hr, pH 8.5) with pancreatic deoxyribonuclease, snake venom diesterase, and 5'-nucleotidase (Worthington Corp.). The acid-soluble portion was fractionated on paper electrophoresis (0.04 *M* citrate buffer, pH 4.5). The radioactive band was eluted and purified by column chromatography (Dowex 50 H^+) and paper electrophoresis (0.04 *M* citrate buffer, pH 3.5). The resulting radioactive material was free of the naturally occurring deoxy- and ribonucleosides as shown by paper chromatography with butanol-borate solvent.

(6) *Enzymes: Escherichia coli* alkaline phosphatase, pancreatic DNase and RNase, and snake venom phosphodiesterase were obtained from Worthington Corp.; rattlesnake venom from the Ross Allen Reptile Institute; crystalline yeast hexokinase from Nutritional Biochemical Corp. RNA polymerase was prepared and assayed according to Chamberlin and Berg.[10] Polynucleotide phosphorylase was prepared by the method of Singer and Guss.[11] Myokinase, phosphoenolpyruvate kinase were purchased from Calbiochem., Inc. L-myosin was the gift of Dr. M. Baranyi. The conditions of enzyme incubation were as follows: (*a*) myokinase, 100 μg enzyme protein/ml, pH 8.5, 5.0 mM $MgCl_2$, 26°, 20 min; (*b*) phosphoenolpyruvate kinase, 100 μg enzyme protein/ml, pH 8.5, 5.0 mM $MgCl_2$, 37°, 15 min; (*c*) pancreatic DNase, 300 μg enzyme protein/ml, pH 7.5, 10 mM $MgCl_2$, 37°, 60 min; (*d*) phosphodiesterase from snake venom, 100 μg enzyme protein/ml, pH 8.5, 5.0 mM $MgCl_2$, 37°, 60 min; (*e*) 5'-nucleotidase from snake venom, 200 μg enzyme protein/ml, pH 8.5, 5.0 mM $MgCl_2$, 37°, 60 min; (*f*) RNA polymerase, as in ref. 10; (*g*) polynucleotide phosphorylase, as in ref. 11; (*h*) pancreatic ribonuclease, 50 μg enzyme protein/ml, pH 6.5, 37°, 30 min; (*i*) myosin ATPase, 2 mg enzyme protein/ml, pH 9, 10 mM $CaCl_2$, 37°, 15 min; (*j*) hexokinase, 15 μg enzyme protein/ml, pH 8, 5 mM, $MgCl_2$, 30°, 15 min.

(7) *Characterization of incorporated tubercidin:* Cells from monolayer or spinner cultures exposed to different concentrations of Tu for varying periods were harvested by centrifugation and washed twice with ice-cold saline. They were then extracted with acid [0.5 *N* $HClO_4$ or 10% trichloroacetic acid (TCA)], washed three times with acid, then with alcohol-ether (3:1) and ether. The components of the acid-soluble fractions were further examined by paper and column chromatography and by paper electrophoresis. The acid-insoluble fraction was dissolved and incubated in KOH (0.5 *N*, 37°, 18 hr); the precipitate which formed on acidification of the alkaline solution was considered to contain the cellular DNA, whereas the material which remained soluble after alkaline hydrolysis was assumed to represent the degraded cellular RNA. (In some experiments the cellular nucleic acids were isolated by the phenol method from intact cells or from cells fractionated by the method of Harris[12] and selectively degraded by chemical and/or enzymatic procedures.) The DNA fraction was degraded by acid (0.5 *N* $HClO_4$, 70°, 15 min) or by a combination of DNase and snake venom phosphodiesterase. The alkaline hydrolysate was further characterized by column and paper chromatography and paper electrophoresis with appropriate reference compounds both before and after enzymatic digestion (with 5'-nucleotidase from rattlesnake venom and alkaline phosphatase from *E. coli*). When the nucleic acids were isolated separately, they were digested with RNase and DNase, respectively, and/or with snake-venom diesterase.

Tubercidin nucleosides were chromatographed or purified by adsorption to Dowex-50 H^+ and elution with 6 *N* NH_4OH. Paper electrophoresis of nucleotides and nucleosides was conducted

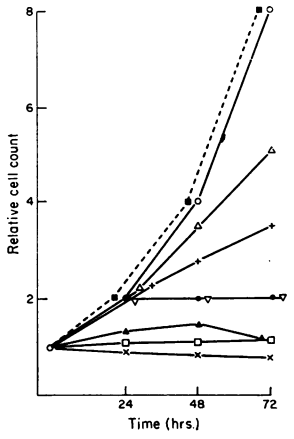

FIG. 2.—Effect of tubercidin and related compounds on the growth of L-cell monolayers. (■——■), 4-amino pyrrolo (2,3-d) pyrimidine, 10 μg/ml; (○——○), control; (Δ——Δ), tubercidin, 0.002 μg/ml; (+——+), 0.01 μg/ml; (●——●), 0.02 μg/ml; (□——□), 0.05 μg/ml; (×——×), 10.0 μg/ml; (▽——▽), deaminated tubercidin, 4 μg/ml; (▲——▲), deoxytubercidin, 1.9 μg/ml.

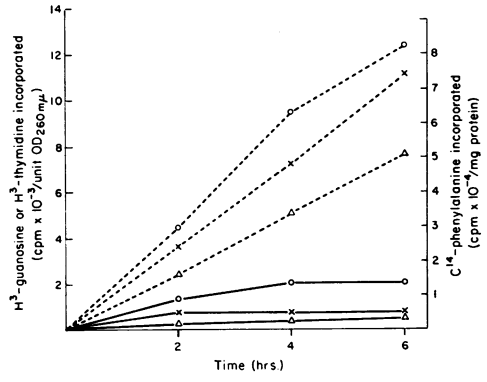

FIG. 3.—Effect of tubercidin (1 μg/ml) on biopolymer formation in suspension cultures of L-cells. Synthesis of DNA, RNA, and protein was measured by the incorporation of H^3-thymidine, H^3-uridine, and C^{14}-L-phenylalanine, respectively. The conditions of incubation and the processing methods used were as in ref. 6. Tubercidin was added 15 min before the respective radioactive precursors. Thymidine incorporation into DNA with (Δ——Δ) and without (Δ- - -Δ) Tu. Uridine incorporation into RNA with (○——○) and without (○- - -○) Tu. Phenylalanine incorporation into protein with (×——×) and without (× - - - ×) Tu.

in a Durrum apparatus at 300 v for 16 hr at 4°, with the following buffers: 0.05 *M* citrate pH 3.5 or 4.5. Paper chromatography (Whatman no. 1, ascending) was performed with isobutyric acid-0.5 *N* NH_4OH (5:3) and in saturated Na-borate n-butanol (14:86) as solvent systems.

The Rf values and adenosine derivatives in the isobutyric acid-ammonia system are as follows: Tu, 0.79; Desamino Tu, 0.55; Deoxy Tu, 0.81; Adenosine, 0.80; Deoxyadenosine, 0.86; Tu 5′ MP, 0.54; 5′ AMP, Tu 5′ DP, 0.36; ADP, 0.34; Tu TP, 0.25; ATP, 0.19.

Results.—(A) *Effects of tubercidin on mouse fibroblasts:* (1) *Inhibition of growth by tubercidin:* The data in Figure 2 show that tubercidin inhibits the growth of L-cells at quite low concentrations. Cells exposed to 1 μg/ml Tu lose colony-forming ability irreversibly within 1 hr. This loss of reproductive viability cannot be restored by subsequent addition of adenosine, inosine, or deoxyadenosine as found also by Owen and Smith.[13] Such cells become rounded and develop cytoplasmic vacuolation and nuclear shrinkage a few hours after treatment with Tu; they then begin to detach from the surface of the Petri dish. These cytologic changes due to tubercidin do not appear in the presence of actinomycin.

(2) *Growth inhibition by desaminotubercidin:* This derivative of tubercidin is less toxic than the parent compound (Fig. 2) and inhibits the growth at the concentration tested only after a lag period of 24 hr.

(3) *Growth inhibition by deoxytubercidin:* The 2′-deoxy derivative of Tu also inhibits cell growth irreversibly, but less effectively than the corresponding riboside. Cells exposed to 1.9 μg/ml of this compound for 48 hr are irreversibly inhibited, but remain attached to the surface of the Petri dish. The cells continue to enlarge in the manner characteristic of cells which have been irradiated or exposed to inhibitors of DNA synthesis,[14] and they do not develop the toxic appearance of cells exposed to

TABLE 1

EFFECT OF TUBERCIDIN ON VIRUS MULTIPLICATION IN L-CELLS

Virus	Tubercidin, μg/ml	Incubation, hr	Cells/plate	Virus/plate	Virus/cell
Vaccinia	None	0	2×10^5	5×10^4	0.25
	None	24		6×10^6	30
	1	24		5×10^4	0.25
Mengovirus	None	0	2×10^5	1.1×10^5	0.55
	None	24		1.2×10^8	600
	10	24		9.6×10^4	0.48
	1	24		6.0×10^4	0.30
	0.1	24		9.1×10^4	0.45
Reovirus	None	0	2×10^5	1.2×10^5	0.6
	None	24		1.5×10^7	75
	10	24		6×10^4	0.3
	1	24		3×10^5	1.5
	0.1	24		1.2×10^5	0.6

Viruses grown and assayed as in *Methods*. The multiplicities of infection and periods of adsorption were vaccinia, 10 (1 hr); Mengovirus, 100 (1 hr); Reovirus, 100 (3 hr). 37°.

Tu. These observations suggest that deoxytubercidin may be a more useful chemotherapeutic agent than Tu itself.

(4) *Effect of amino pyrrolo (2,3-d)pyrimidine on L-cells:* The aglycone of Tu is not toxic to L-cells and does not inhibit cell division, even when present in the culture medium at 10.0 μg/ml for 72 hr. This finding is not surprising since this compound, originally synthesized some years ago,[15] is inactive in other systems. The inertness of the aglycone must be attributed to the failure of its conversion to nucleoside or nucleotide by the appropriate cellular synthesizing enzymes.

(5) *Inhibition of viral growth by tubercidin:* The data in Table 1 show that the growth of the DNA-virus, vaccinia, is inhibited by tubercidin. The growth of Reovirus III, whose RNA is double-stranded,[16] and of Mengovirus, whose RNA is single-stranded,[17] are also suppressed by Tu.

(6) *Inhibition of cellular macromolecule synthesis by tubercidin:* The ability of Tu to inhibit cellular synthesis of DNA, RNA, and protein was studied to obtain further insight into the lethal properties of this antibiotic. The data in Figure 3 show that the synthesis of the three biopolymers in question is inhibited by tubercidin. It is not surprising that the syntheses of DNA and RNA are inhibited very rapidly; however, the relatively rapid interruption of protein synthesis is somewhat unexpected since actinomycin, which inhibits RNA synthesis even more effectively than Tu, allows protein synthesis to proceed for longer periods.[6] Although Tu is incorporated into ribosomal as well as into soluble RNA, the rapidity of suppression of protein synthesis suggests that Tu may interfere with the function of soluble RNA and/or amino-acid-activating enzymes.

(B) *Metabolism of tubercidin in L-cells:* (1) *Incorporation of tubercidin into cellular nucleic acids:* When radioactive Tu is added to fibroblast cultures, intracellular radioactivity accumulates rapidly in both acid-soluble and acid-insoluble fractions. At a level of 1 μg/ml Tu, 50 per cent of the antibiotic is usually concentrated by the cells within 4 hr; of this, approximately 80–90 per cent are found in the acid-soluble fraction, entirely as ribonucleotides; 5–10 per cent are in the acid-insoluble fraction. A typical balance sheet from one experiment was: medium, 39.2%; intracellular acid-soluble, 55%; lipid fraction, 0.1%; DNA fraction, 1.4%; RNA fraction, 3.8%.

The acid-insoluble form of Tu was characterized as follows: (*a*) the radioactive

material released on alkaline hydrolysis closely resembled 2′(3′) AMP chromatographically and electrophoretically; its properties were not altered by 5′-nucleotidase; it was converted by *E. coli* phosphatase to a substance which was chromatographically and electrophoretically indistinguishable from authentic Tu, and its glycosidic bond showed the acid resistance of authentic Tu. Incubation of the acid-insoluble fraction with excess RNase (37°, 2 hr) solubilizes all the radioactivity which can be released by alkaline hydrolysis. It is concluded that tubercidin is incorporated into RNA as the ribonucleotide. Since 95 per cent of the radioactivity released by alkali and by RNase was in nucleotide form, only a small fraction of the incorporated Tu was in nucleoside terminal position. When the RNA which had been isolated by the phenol method[18] from ribosomes and/or the soluble supernatant fraction (following centrifugation of cell extracts for 2 hr at 105,000 × *g*) was digested with venom diesterase, the release of acid-soluble radioactivity paralleled that of UV-absorbing material. Therefore, Tu is present throughout polyribonucleotide chains. (*b*) Digestion with DNase and phosphodiesterase of the acid-insoluble residue remaining after alkaline hydrolysis released a radioactive nucleotide whose chromatographic and electrophoretic behavior resembled that of 2′-deoxyadenylic acid. The radioactive nucleotide was converted by 5′-nucleotidase and alkaline phosphatase to a product with the following characteristics: mobility on electrophoresis in citrate buffer pH 3.5 identical with Tu; mobility in chromatography in butanol-borate corresponded to Rf 0.78, that of Tu being 0; acid hydrolysis yielded a product chromatograpically indistinguishable from authentic 4-amino pyrrolo (2,3-d) pyrimidine, the aglycone of Tu. The acid lability of the glycosidic bond of the nucleotide released from DNA was intermediate between that of TuMP and 2′-deoxy AMP (Fig. 4). It is concluded that Tu is transformed to a 2′-deoxy derivative intracellularly and incorporated in this form into DNA.

(2) *Effect of inhibitors on incorporation of Tu into nucleic acids:* The data in Table 2 show that 5-fluorodeoxyuridine (FUDR), an inhibitor of thymidylate synthetase[19] and of DNA synthesis,[20] suppresses the incorporation of Tu into DNA. After removal of both FUDR and Tu, a substantial incorporation of Tu into DNA is observed even when the cells are provided with a tenfold excess of adenosine. This incorporation into DNA also occurs when the cells are incubated with FUDR before and after, as well as during, the period of exposure to Tu. When FUDR is removed 4 hr after Tu, Tu is rapidly incorporated into DNA for a succeeding period of 4 hr even in the presence of excess adenosine. Thus, the intracellular pool of Tu, or its nucleotide derivatives, is sufficient to provide Tu for deoxynucleotide conversion whenever the cell is permitted to synthesize DNA. It is also seen that actinomycin, which inhibits the DNA-dependent synthesis of RNA that accounts for

TABLE 2

THE EFFECT OF INHIBITORS ON THE INCORPORATION OF H^3-TUBERCIDIN INTO NUCLEIC ACIDS

Addition	cpm/unit O.D.$_{260}$ mμ RNA	cpm/unit O.D.$_{260}$ mμ DNA
None	31,224	22,646
FUDR 10^{-6}	30,949	1,326
FUDR 10^{-6} + thymidine 10^{-5}	30,676	17,396
Actinomycin, 1 μg/ml	2,647	15,496

A suspension culture of fibroblasts (1×10^6/ml) was incubated with Tu (1 μg/ml; 8×10^5 cpm/μg) for 2 hr. The incorporated radioactivity was determined on the cells harvested from 5 ml of culture as described in *Methods*. The inhibitors, where present, were added simultaneously with Tu.

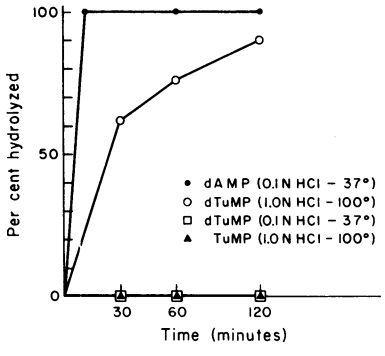

FIG. 4.—Acid lability of nucleotides. Purified radioactive nucleotides were incubated under the conditions shown. Aliquots were removed at timed intervals, and the percentage of radioactivity migrating as the original nucleotide was determined.

formation of all cellular RNA fractions,[6] suppresses the incorporation of Tu into RNA. This incorporation is, therefore, mediated by the usual cellular pathways of RNA synthesis.

(3) *Incorporation of Tu into virus-specific RNA:* Because inhibition by Tu of Mengovirus growth occurs in the presence of actinomycin, Tu, or its nucleotide derivatives, probably interferes directly in some way with viral functions, and it appears desirable to determine whether Tu becomes incorporated into virus-specific RNA. To investigate this possibility we have made use of the fact that actinomycin interferes with cellular, but not with Mengovirus RNA synthesis[6] so that the synthesis of viral RNA may be studied in actinomycin-treated, infected cells. The data in Figure 5 show that Tu is indeed incorporated into virus-specific RNA. Thus, the virus RNA-polymerizing system, like that of the uninfected cell, can utilize Tu-nucleotides as precursors for polynucleotide synthesis.

(4) *Metabolism of desaminotubercidin:* When radioactive desaminotubercidin is added to fibroblast cultures, radioactivity accumulates intracellularly in both acid-soluble and acid-insoluble fractions. However, the amount metabolized in this way is much smaller than in the case of Tu. By appropriate enzymatic and chemical degradation and chromatographic characterization (see *Methods*), it was established that the acid-insoluble radioactivity consists exclusively of Tu derivatives; that is, desamino-Tu is reaminated before incorporation. The acid-soluble fraction contained phosphorylated derivatives of desamino-Tu and of Tu. Thus, the growth inhibitory effect of desamino-Tu probably derives from its conversion to Tu or Tu derivatives at some stage of its metabolism.

(5) *Metabolism of 2′-deoxy-Tu:* Cultures incubated in the presence of radioactive 2′-deoxy-Tu incorporate radioactivity into DNA but not into RNA. However, the incorporation into DNA is appreciably less than when Tu, the corresponding riboside, is the precursor; the basis of this difference has not been elucidated. The intracellular acid-soluble fraction of cells exposed to 2′-deoxy-Tu consisted entirely of the free nucleoside—no phosphorylated derivatives were detected.

(6) *Characterization of the components of the acid-soluble pool:* When the acid-soluble fraction obtained from L-cells exposed to H^3-Tu is examined by paper electrophoresis, radioactivity is observed in three bands. The first of these (I), whose electrophoretic mobility is close to that of 5′ AMP, accounts for 5–10 per cent of the total radioactivity. Treatment of I with 5′-nucleotidase yields a radiooctive product chromatographically and electrophoretically indistinguishable from Tu; I is therefore Tu-5′-monophosphate (Tu-5′ MP).

The material in the second band (II) which accounts for 8–15 per cent of the total radioactivity is converted to I by 7-min acid hydrolysis. Treatment of II with myokinase (Fig. 6) yields I, II, and III, and with phosphoenolpyruvate kinase and

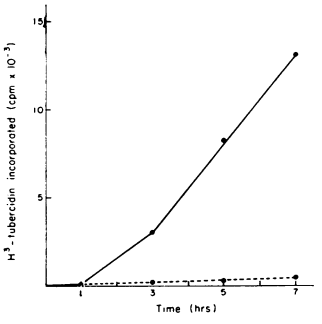

FIG. 5.—Incorporation of H^3-tubercidin into the RNA of L-cells infected with Mengovirus. A suspension culture (4×10^5/ml) of L-cells was incubated with actinomycin (2 μg/ml) for 60 min. The cells were concentrated to 5×10^6/ml, divided into two portions, one of which was infected with Mengovirus at a multiplicity of 50. Following a period of virus adsorption (60 min) the cells from both portions were collected by centrifugation, suspended, and incubated in fresh medium at 5×10^5/ml. Actinomycin (2 μg/ml) was present throughout the experiment. H^3-tubercidin was added 4 hr after exposure to the virus. Aliquots were removed at the indicated times, and the radioactivity of the RNA (alkali-labile fraction of the acid-washed, defatted cells) determined in a scintillation counter. Infected (●——●); uninfected (●- - -●).

an excess of phosphoenolpyruvate produces a quantitative transformation to III. We conclude that II must be Tu-5′-diphosphate (Tu-5′-DP).

The third band (III) contains most of the radioactivity (75–85%) of the acid-soluble fraction. When III is reacted with crystalline yeast hexokinase in the presence of glucose or with Ca^{++}-activated L-myosin, it is quantitatively converted to II. It is concluded that III represents Tu-5′-triphosphate (Tu-5′ TP).

(7) *Miscellaneous enzymatic reactions involving Tu-nucleotide:* (*a*) RNA polymerase has been tested for the ability to use as a substrate chemically synthesized and biosynthetic TuTP. Both forms of TuTP could replace ATP for enzymatic RNA synthesis, and TuTP functioned efficiently in heteropolymer as well as homopolymer formation. (*b*) Purified RNA containing radioactive Tu was treated with polynucleotide phosphorylase (1 mg) in the presence of 0.05 *M* phosphate. After 15 min, 38 per cent of the radioactivity in RNA was converted to acid-soluble form, the major component of which was identified as TuDP on the basis of chromatographic and electrophoretic behavior.

Discussion.—The data presented above show that Tu and its derivatives can substitute effectively for the corresponding adenosine compounds in a surprisingly wide variety of cellular and enzymatic reactions. To our knowledge, Tu is unique among analogues in the extent of its incorporation into both RNA and DNA. This suggests that the substrate specificity of enzymes which metabolize adenine nucleotides only rarely involves the N-7 position of the purine. However, the finding that neither Tu, deoxy-Tu, nor desamino-Tu is biosynthetically converted to the corresponding guanine analogues at a detectable rate suggests that the specificity of a few of these enzymes may be based on the imidazole portion of the purine. It would be of interest to investigate the extent to which Tu could substitute for adenosine in the synthesis and functioning of coenzymes, of S-adenosyl methionine, and as a phosphate acceptor in oxidative phosphorylation.

The lethal effect of Tu on L-cells is probably due to its incorporation into nucleic acids. The incorporation of deoxy-Tu into DNA alone is associated with irreversible loss of viability. We have not succeeded in directing the incorporation of Tu into cellular RNA alone, so that no definitive appraisal of the consequences of this substitution can be made. Nevertheless, it can be assumed that the presence of Tu even in RNA alone would be extremely deleterious for nucleic acid function since (*a*) RNA virus growth is totally suppressed even by very low concentrations of Tu; (*b*) the higher cytotoxicity of Tu as compared with that of deoxy-Tu probably reflects incorporation into RNA rather than a mere increase in the efficiency of up-

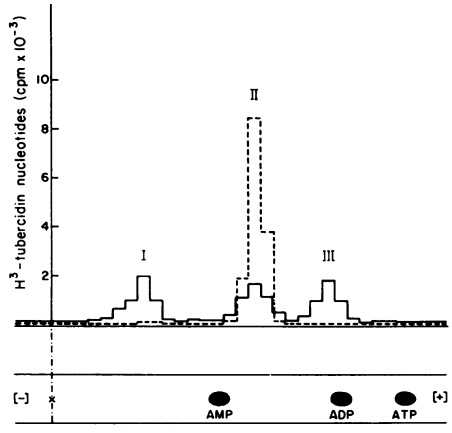

FIG. 6.—Distribution of radioactivity on electrophoresis of Tu nucleotides. Fraction II of the acid-soluble intracellular material before (- - - - -) and after (———) treatment with myokinase. ■ The electrophoretic mobility of the corresponding adenosine derivatives is shown for comparison.

take into DNA alone; (*c*) actinomycin prevents the extreme cytological changes normally produced by Tu toxicity but does not suppress incorporation of Tu into DNA.

The implications of Tu incorporation for the structure and function of helical nucleic acids are of some interest. The N-7 of purines is located in the major groove of helical polynucleotides. Its replacement by C-H, as occurs in Tu, eliminates an effective potential acceptor of hydrogen for H bonding in favor of a grouping which is unlikely to participate in H bonding. In addition to this substitution, Tu introduces a proton into the major groove at a position where none is present normally. These considerations suggest that polynucleotides containing Tu may be useful for investigating the template functions of nucleic acids and the mechanism of the binding of small molecules, such as diamines, which have AT specificity.[21] Such polynucleotides may also be of some value in testing current formulations of the structures of poly A + poly U (1:2) and poly A + I (1:2) helices, and of the helical form of poly A, in all of which H bonding involving position 7 is considered to play an important role.[22–25] Current efforts are therefore being directed to the enzymatic synthesis and to the study of such polymers.

We thank E. L. Tatum for encouragement, and the Muscular Dystrophy Associations of America, the Helen Hay Whitney Foundation, the American Cancer Society (grant P-299A), and the National Institutes of Health (grant GM 10717-02) for support.

Abbreviations: AMP, ADP, ATP—5′-monophosphate, diphosphate, and triphosphate of adenosine; poly A, poly U, poly I—polyadenylic, polyuridylic polyinosinic acids, respectively; RNA, DNA—ribonucleic, deoxyribonucleic acid; dAMP—2′-deoxyadenosine-5′-monophosphate.

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*ON THE RNA SYNTHESIZED DURING THE LAMPBRUSH PHASE OF AMPHIBIAN OÖGENESIS**

By ERIC H. DAVIDSON, V. G. ALLFREY, AND A. E. MIRSKY

THE ROCKEFELLER INSTITUTE

Communicated June 8, 1964

Lampbrush chromosomes have been observed in the growing oöcytes of animals ranging from mollusks to mammals. They are present throughout the diplotene of the first meiotic division. These elongate, paired structures bear many thousands of loops projecting laterally from the main chromosomal axis, and they are characteristically accompanied in the nuclear sap by several thousand small nucleoli. It was long ago suggested that the organization of the egg and the early processes of embryogenesis were the result of nuclear activity occurring during ovarian oögenesis,[1] and we now know the lampbrush chromosomes of amphibian oöcytes are in a state of intense genetic activity. The visible evidence of this activity is provided by autoradiographic studies of RNA synthesis, which have demonstrated newly synthesized RNA in all regions of the chromosomes[2, 3] as well as in the nucleoli. A significant comparison has been drawn by Izawa, Allfrey, and Mirsky to illustrate the unique, high level of genic activity characteristic of lampbrush chromosomes.[4] Their measurements show that the ratio of RNA to DNA in lampbrush chromosomes is over 100 times greater than the comparable figure for the chromatin of a typical differentiated cell such as a liver cell. The experiments to be reported here throw some light on the nature of this intense gene activity. It is shown that most or all of the RNA synthesized during the lampbrush stage of oögenesis in the anuran *Xenopus laevis* is conserved, at least until ovulation, and that over 90 per cent of this RNA is of ribosomal type. The remaining RNA synthesized in these oöcytes appears to be of a different type, and possesses unusual characteristics.

Materials and Methods.—Total acid-insoluble RNA: Mature *Xenopus* females were injected with pituitary extract to stimulate oögenesis, and three days later the RNA of the oöcytes was labeled by injection into the dorsal lymph sac of a total of 1 mC of uridine-H^3/animal, administered in three equal doses over the succeeding 3 days. Three days following the termination of labeling, the ovaries were removed, and oöcytes of the desired stages were dissected free from their follicles with watchmaker's forceps, under Barth and Barth's solution X,[5] containing 2 × NaCl. It is essential that the follicles be removed, since autoradiographs of Ficq and others have shown that follicle cells are very active in RNA synthesis.[6, 7] The oöcytes were washed in cold acid and fat solvents, and the RNA extracted with hot 0.5 *N* PCA.[8] RNA content was