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Utilization of Doubly Labeled Pyrimidine Ribosides and Deoxyribosides by the Rat

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Cytidine, uridine, deoxycytidine and thymidine were prepared from ^{14}C-labeled polynucleotides. The doubly labeled nucleosides were separately injected into partially hepatectomized rats and their incorporation into the pyrimidine part and the sugar moiety of polynucleotide pyrimidines was studied.

The intact incorporation of each administered labeled compound into the corresponding nucleoside was demonstrated. After injection of the labeled pyrimidine ribosides the ratio between the specific activities of pyrimidine and deoxyribose of the isolated deoxyribosides was very close to the corresponding pyrimidine/ribose ratio of the precursor, indicating a relatively direct conversion of the ribosides to deoxyribosides. No utilization of deoxyribosides for the synthesis of ribosides was observed.

In no case did the purine ribosides contain significant activity.

^{15}N-labeled pyrimidine ribosides and deoxyribosides are incorporated by the rat into polynucleotide pyrimidines [1,2]. Free pyrimidines, however, are very poorly or not at all utilized [3–7]. This was taken as evidence that the labeled nucleosides were incorporated as intact molecules into both pentose nucleic acid (PNA) and deoxypentose nucleic acid (DNA) [1,2]. Rose and Schweigert [8] subsequently demonstrated that the ribose and pyrimidine of doubly labeled cytidine-^{14}C were incorporated to the same extent into the pyrimidine and carbohydrate moiety of PNA and DNA pyrimidine nucleosides. This established that the postulated intact incorporation of the labeled nucleosides had taken place at least with cytidine.

In the present investigation cytidine, uridine, deoxycytidine and thymidine were prepared from over all ^{14}C-labeled polynucleotides. The nucleosides, which were accordingly labeled in both the pyrimidine ring and the carbohydrate moiety, were injected into partially hepatectomized rats, and their utilization for polynucleotide biosynthesis in the liver and the intestine was studied.

EXPERIMENTAL

Preparation of labeled ribosides. ^{14}C-yeast nucleic acid was purchased from Schwarz laboratories, Inc. After alkaline hydrolysis the labeled mononucleotides were prepared by gradient elution chromatography on a Dowex-2 formate column [9]. The pyrimidine nucleotides were dephosphorylated with prostatic phosphatase and the formed ribosides were then prepared by starch chromatography [10].

Preparation of labeled deoxyribosides. Labeled DNA was obtained by extraction [11] from *E. coli* grown in the presence of lactate-3-^{14}C *. Labeled deoxynucleotides were prepared by the combined action of deoxyribonuclease and snake venom diesterase [12]. After chromatography [9] the pyrimidine deoxynucleotides were dephosphorylated with snake venom monoesterase and the formed deoxycytidine and thymidine were chromatographed on starch [13].

The purity of all the labeled compounds used for the animal experiments was checked from light absorption data in the ultraviolet [14] and paper chromatography [15].

Before use each labeled compound was diluted with the corresponding nonlabeled riboside or deoxyriboside. The ratio between the specific activities of the pyrimidine ring and the carbohydrate was determined in duplicate experiments as described below.

Animal experiments. Rats weighing 150—200 g were subjected to partial hepatectomy according to the technique used in this laboratory [16]. Starting 20 h after the operation each nucleoside, dissolved in 0.9 % NaCl, was injected into one rat at two hours interval. A total of five injections (0.5 ml each) was given to each rat. The rats were killed 42 h after the operation, the livers and the thoroughly washed small intestines were removed and separately placed in alcohol.

Isolation of polynucleotide compounds for ^{14}C-determinations. PNA and DNA were prepared and separated according to Hammarsten [11]. The ribosides and deoxyribosides, respectively, were obtained by a combination of ion exchange and starch chromatography as outlined above for the preparation of the isotopic compounds.

Degradation of nucleosides. In order to determine the ratio between the specific activities of the pyrimidine ring and of the sugar the following procedure was adopted:

Each nucleoside was dissolved in 5 ml of water. The amount of substance was measured by light absorption [14] after dilution of aliquots with 0.1 N HCl. Radioactivity was measured on infinitely thin samples (less than 0.2 μmoles/cm^2) in a Tracerlab Sc-18 windowless flow counter and the specific activity of the whole nucleoside was calculated.

The rest of the pyrimidine nucleoside containing solution was evaporated to dryness *in vacuo*. The residue was transferred to a bomb tube with a small amount of conc. formic acid (1—2 ml) and hydrolyzed at 175° [17], deoxyribosides for 1 h, ribosides for 3 h. This treatment liberated the free pyrimidines. The only nucleoside which was not quantitatively split was uridine. The formic acid was removed by repeated evaporation *in vacuo*, the residue was dissolved in butanol-water and chromatographed on starch [18].

The specific activities of the pyrimidines obtained in this way was calculated after light absorption measurements and determinations of radioactivity as described for the nucleosides.

This method gave the specific activity of the sugar moiety as the difference between that of the nucleoside and that of the pyrimidine ring.

RESULTS

Cytidine-^{14}C. Three different animal experiments were carried out and are recorded in Table 1. In accordance with earlier observations [1,8] cytidine was incorporated into all pyrimidines of both PNA and DNA. The ratios between the specific activities of the pyrimidine ring and of the carbohydrate moiety showed some variations for each nucleoside in the different experiments probably largely depending on the fact that the specific activity of the sugar was determined by difference. The average ratio for all cytidines from both

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Table 1. Incorporation of doubly labeled cytidine-^{14}C into polynucleotides of the rat.
The specific activity of the precursor was 172 000 ct/min/μmole. The ratio between the specific activities of ribose and cytosine was 1.85.
Each rat received a total of 10 μmoles of cytidine in expts. 1 and 2, 5 μmoles of cytidine in expt. 3.

	Liver				Intestine			
	counts/min/μmole			specific act. of sugar / specific.act.of pyrim.	counts/min/μmole			specif. act. of sugar / specific act. of pyrim.
	Experiment No. 1	2	3		Experiment No. 1	2	3	
PNA:								
Cytidine	1 950	1 520	1 110		1 520	2 480	1 420	
Cytosine	660	540	440	1.95; 1.81; 1.53	550	840	570	1.77; 1.85; 1.49
Ribose *	1 290	980	670		970	1 640	850	
Uridine	500	330	250		520	700	420	
Uracil	200	140	100	1.50; 1.36; 1.50	200	250	170	1.60; 1.80; 1.47
Ribose *	300	190	150		320	450	250	
DNA:								
Deoxycytidine	1 340	1 200	720		1 070	750	610	
Cytosine	550	490	240	1.44; 1.45; 2.00	410	310	230	1.61; 1.42; 1.65
Deoxyribose *	790	710	480		660	440	380	
Thymidine	330	280	160		130	220	195	
Thymine	140	110	65	1.36; 1.55; 1.46	50	90	75	1.60; 1.44; 1.60
Deoxyribose *	190	170	95		80	130	120	

* Calc. by difference

liver and intestinal polynucleotides, however, was clearly very close to the corresponding value in the administered precursor.

The uracil/ribose ratios in the polynucleotide uridines were slightly lower than the corresponding values for cytidine. This was also true for the two deoxyribosides. The incorporation into the purine nucleosides is not recorded in the table, since it was insignificant in all cases (less than 10 counts/μmole).

Uridine-^{14}C. Relatively large amounts of the isotopic riboside were administered in order to obtain high enough counts in the isolated compounds (Table 2). In this experiment the pyrimidine/ribose ratios in the PNA ribosides were quite close to that of the administered precursor, and this was also true for the pyrimidine/deoxyribose ratios. Again only very low counts were found in the purine compounds.

Labeled deoxyribosides. The results of experiments with deoxycytidine-^{14}C and thymidine-^{14}C are demonstrated in Table 3. The former compound was incorporated into both deoxycytidine and thymidine of DNA, while the latter was utilized exclusively for thymidine synthesis. In no case was there significant incorporation into PNA pyrimidine ribosides or purine compounds.

Degradation of the isolated deoxyribosides showed that in all cases pyrimidine and deoxyribose were incorporated to about the same extent into DNA.

Table 2. Incorporation of doubly labeled uridine-^{14}C into polynucleotides of the rat. 100 μmoles of uridine (71 000 cts/min/μmole) were injected into one rat. The ratio between the specific activities of ribose and uracil was 1.46.

	Liver		Intestine	
	counts/min/ μmole	spec. act. of sugar / spec. act. of pyrim.	ct/min/ μmole	spec. act. of sugar / spec. act. of pyrim.
PNA:				
Cytidine	320		330	
Cytosine	130	1.38	120	1.75
Ribose *	180		210	
Uridine	380		330	
Uracil	150	1.53	140	1.36
Ribose *	230		190	
DNA:				
Deoxycytidine	290		130	
Cytosine	125	1.32	60	1.17
Deoxyribose *	165		70	
Thymidine	220		110	
Thymine	105	1.10	45	1.45
Deoxyribose *	115		65	

* Calc. by difference.

DISCUSSION

It is quite clear from the results that the rat has the capacity to incorporate pyrimidine ribosides and deoxyribosides into tissue polynucleotides without a more pronounced breakage of the pyrimidine-sugar bond.

Most experiments were carried out with labeled cytidine, since this compound, when administered in small amounts, was incorporated best into all pyrimidine ribosides and deoxyribosides. The fact that the ratio between the specific activities of cytosine and of ribose within experimental limits was the same in the injected precursor and in the isolated polynucleotide cytidine demonstrates the intact incorporation of the precursor. Such a finding would be extremely improbable if the glycoside linkage of the injected compound were first split and the split products were subsequently utilized for polynucleotide synthesis.

The uracil/ribose ratio was in all cases slightly lower than the cytosine/ribose ratio. Unless this finding depends on some unknown systematic experimental error, it might indicate that a small amount of pyrimidine was incorporated into uridine without being attached to ribose.

The pyrimidine/deoxyribose ratios in the isolated deoxycytidines and thymidines were on the average slightly lower than the pyrimidine/ribose ratios in the isolated ribosides. The difference was, however, not very significant. It is clear that the main route for the incorporation involved a reduction of the

Table 3. Incorporation of doubly labeled deoxyribosides into polynucleotides of the rat. In two experiments 25 μmoles of each deoxyriboside (deoxycytidine = 13 500 ct/min/μmole; thymidine = 13 200 ct/min/μmole) were injected into one rat. The ratios between the specific activities of pyrimidine and deoxyribose were for deoxycytidine 0.97 and for thymidine 0.99.

Isolated	Deoxycytidine injected				Thymidine injected			
	Liver		Intestine		Liver		Intestine	
	ct/min/μmole	sp.act. sugar / sp.act pyrim.	ct/min/μmole	sp.act. sugar / sp.act. pyrim.	ct/min/μmole	sp.act. sugar / sp.act. pyrim.	ct/min/μmole	sp.act. sugar / sp.act. pyrim.
PNA:								
Cytidine	8		9		6		0	
Uridine	5		6		3		2	
DNA:								
Deoxycytidine	1 120		480		11		7	
Cytosine	510	1.20	250	0.92				
Deoxyribose *	610		230					
Thymidine	375		375		620		375	
Thymine	195	0.92	185	1.03	330	0.88	145	0.89
Deoxyribose *	180		190		290		130	

* Calc. by difference.

ribose of the *intact riboside* molecule or a derivative of it. The results definitely exclude a mechanism by which only a smaller part of the ribose molecule was attached to the pyrimidine during the reduction *, as might be visualized in analogy with known enzyme reactions [19]. Furthermore, transglycosidation mechanisms, as demonstrated for microorganisms [20], are also excluded. In this connection the absence of label from the purine bound ribose is of importance.

The data from the incorporation experiments with labeled uridine, deoxycytidine and thymidine all demonstrate the intact incorporation of the administered nucleosides.

Uridine-^{14}C behaved similar to cytidine-^{14}C and showed the transformation of the intact riboside to all polynucleotide pyrimidine nucleosides. Roll *et al.*[21] found some preferential incorporation of uracil from over all labeled uridylic acid-^{14}C into deoxycytidylic and thymidylic acids of the mixed internal organs of the rat. Our data do not show a more pronounced effect of this kind.

The results with the ^{14}C-labeled deoxyribosides confirm the conclusions drawn from earlier experiments with ^{15}N-labeled deoxyribosides that the conversion of ribosides to deoxyribosides *in vivo* is essentially irreversible.

* The loss of one carbon atom of the ribose chain during the reduction is not excluded from the results.

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