EXCRETION OF 5-RIBOSYLURACIL (PSEUDOURIDINE) BY HEALTHY PERSONS AND CANCER PATIENTS BEFORE AND AFTER RADIOTHERAPY

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Pseudouridine (5-ribosyluracil) was first discovered in yeast RNA in 1957 [3] and later (1960) in human urine [2]. It has been established that pseudouridylic acid is a minor component of messenger RNA [5, 6, 9, 13]. One difference between this compound and uridine is that the ribose in the pseudouridine is attached, not to nitrogen, but to the position 5 of the pyrimidine ring. This gives an unusual glycoside C—C instead of N—C bond.

The important metabolic role of pseudouridine in the cell, its high chemical stability, and its discovery in human urine determine the need for a study of the excretion of pseudouridine in the urine of healthy persons and of cancer patients before and after radiotherapy.

EXPERIMENTAL METHOD

The content of pseudouridine was determined in the urine of 8 healthy men aged 25-40 years and 8 patients with carcinoma of the esophagus or lung before irradiation and after the 1st and 10th sessions of local x-ray therapy. The total doses were 100 and 1800 R respectively.

The pseudouridine in the urine was estimated by the method described by Eisen and co-workers [7], with a few modifications to the system creating the elution gradient and using other types of ion-exchange resins and solvents for the paper chromatography. The order of the determination was: 1 ml of the urine concentrate was diluted to 10 ml with water and passed for desalting through a 1 × 12 cm column containing the anion-exchange resin Dowex 1 × 10, 100/200 mesh, in the formate form, and washed with 25 ml of a 0.1 N solution of HCOOH. The pooled eluates were passed through a 1 × 2 cm column with the cation-exchange resin Dowex 50B × 4, 200/400 mesh in hydrogen form and again washed with 25 ml of a 0.1 N solution of HCOOH. The eluates obtained were dried in vacuo at 18-20° within the liquid, and the dry residue was dissolved in 5 ml 0.05 N ammonium borate solution (pH 9.2). If after the residue had dissolved the pH of the solution was below 9, a few drops of ammonia were added to bring its value to 9.0. This desalted, alkaline ammonium borate solution, containing derivatives of nicotinamide, uracil, and the two isomeric forms of pseudouridine (B and C), was applied to a 1 × 15 cm column with the anion-exchange resin Dowex 1 × 10, 100/200 mesh, in the formate form, which was washed before chromatography was undertaken with 0.1 N ammonium borate solution to give an alkaline eluate. After the sample had soaked into the resin, it was washed off with 2-3 ml of 0.015 N ammonium borate solution and an elution gradient was created in accordance with the following scheme: 250 ml of 0.015 M ammonium borate solution was poured into a conical mixing flask and 500 ml of a 0.025 M solution of ammonium chloride into a cylindrical vessel. The diameters of the vessels were such that the level of the solutions was at the same height in both. Solution passed from the cylindrical vessel into the mixing flask by the principle of communicating vessels. The speed of elution was 1.5 ml/min and the volume of the siphon was 15 ml. The fractions were collected with an automatic collector. Pseudouridine was identified by the Rf of the stains on the chromatogram (Whatman No. 3 MM paper; solvent: 6 parts N-butanol + 1 part 0.6 M NH_4OH), from the absorption spectra at pH 2.0 and 12.0, and by comparison of λ_{max} and λ_{min} and the ratios between the values of the optical density at different wavelengths: 250/260, 280/260, 290/260, and 300/260.

EXPERIMENTAL RESULTS

A typical elution curve is given in Fig. 1. The last two peaks contained pseudouridine B and C. The absorption spectra of the pseudouridine isolated from the urine at pH 2.0 and 12.0 are shown in Fig. 2. The fractions of

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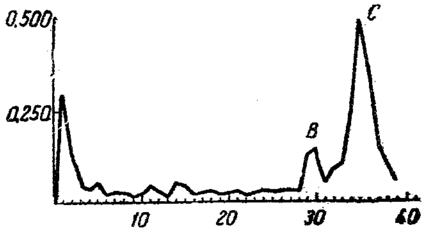

Fig. 1. Optical density (260 mμ) of fractions obtained during chromatography of desalted urine on a column with Dowex 1 × 10, 100/200 mesh, 1 × 15 cm. Volume of fractions 15 ml. Along the axis of abscissas—fraction No., along the axis of ordinates—optical density. Peak B—pseudouridine B; peak C—pseudouridine C.

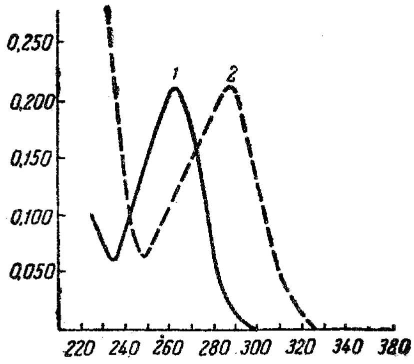

Fig. 2. Absorption spectrum of pseudouridine in the urine. Along the axis of abscissas—wavelength (mμ). Along the axis of ordinates—optical density. 1) pH 2.0; 2) pH 12.0.

Excretion of Pseudouridine in the Urine (in mg/24 h) in Healthy Persons and Cancer Patients before and after Local X-Ray Irradiation

Healthy	Content of pseudouridine	Ill	Content of pseudouridine		
			before irradiation	after irradiation	
				in a dose of 100 R	in a dose of 1800 R
R.	57,5	Sn.	38,6	59,0	—
P.	65,0	K.	59,0	—	104,0
S.	47,7	Ks.	26,3	73,0	125,8
Sk.	66,6	G.	53,0	50,7	144,5
K.	51,0	N.	—	—	82,0
F.	59,4	M.	71,5	84,0	99,5
B.	57,0	S.	—	41,5	—
M.	46,0	Nk.	86,5	—	—
Mean	56,2		55,9	61,6	111,13

the peaks containing pseudouridine were pooled, their pH was adjusted to 7.0, and the value of the extinction at 260 mμ was determined. Taking the coefficient of molar extinction of pseudouridine at pH 7.0 as 8600, the content of pseudouridine was determined in milligrams and the 24-hour excretion in the urine calculated.

The content of the two isometric forms (B and C) of pseudouridine in the healthy men varied relatively little, and the mean of the eight determinations was 56.2 mg/24 h. This is in full agreement with data in the literature [17]. So far as the content of pseudouridine in the cancer patients before irradiation is concerned, in the patients investigated considerable individual variations from the mean value were observed, some lower (26.3 mg/24 h) and some higher (86.5 mg/24 h).

It is difficult to say whether a single session of x-ray irradiation in a dose of 100 R had any effect on the amount of pseudouridine excreted, because of the large individual variations, but the mean value (61.6 mg/24 h) was slightly higher than before irradiation, although the difference lay within the limits of error.

Repeated local irradiation of the chest in a total dose of 1800 R, as is clear from the table, undoubtedly led to an increase in the excretion of pseudouridine from the cancer patients. A significant increase in the content of pseudouridine in this particular case was observed during comparison both of the mean values (61.6 mg/24 h and 111.1 mg/24 h) and of the indices for the individual patients.

To understand the importance of pseudouridine during irradiation, in leukemia and, possibly, in certain forms and stages of cancer, it is necessary to know the fate of the pseudouridine in the body: the source, the place and rate of its biosynthesis and breakdown (if it is broken down), its relationship to the rate of biosynthesis of other pyrimidines, its blood level and its level of excretion in various normal states and pathological conditions. Experiments with labeled uracil, uridine, and orotic acid [18] have led to the conclusion that the main source of the biosynthesis of pseudouridine in the animal and human organism may be orotic acid and uridine. Most likely the main site of the biosynthesis of pseudouridine is in tissues with intensive protein synthesis, such as the pancreas, the hemopoietic tissue, regenerating tissues, and malignant tumors. Pseudouridine is found in the dog's pancreas [10] and the rat's liver [5].

Weissman and co-workers, using labeled C^{14}-pseudouridine, found that the rate of accumulation of maximal radioactivity in the urine, the rate of its decrease, and the absence of radioactive carbon dioxide in the expired air justify the suggestion that pseudouridine cannot be converted into other compounds and is not metabolized, so that it is completely and rapidly excreted in the urine. Its clearance was found to be very high, because labeled pseudouridine could not be detected in the blood even immediately after its administration. These findings support the view that pseudouridine cannot be utilized for RNA synthesis, because no kinase catalyzing its phosphorylation has been found [8].

Eisen and co-workers [7] showed that the excreted pseudouridine is exogenous in origin. The amount of this substance in the urine excreted in a definite time can thus be used as a measure of the rate of its biosynthesis in the organs. Weissman and co-workers [17] suggested a method of calculating the rate of biosynthesis of all the pyrimidine substances of the organism, based on the amount of excreted pseudouridine.

The observations on the cancer patients were confirmed by the authors' experiments on animals (in the press) and by a short communication by Drahovsky [4]. The increase in the excretion of pseudouridine during irradiation cannot be regarded as an increase in the biosynthesis of the pyrimidines, because irradiation not only leads to the early and marked depression of the incorporation of the various pyrimidine precursors [11], but also sharply depresses the activity of the enzymes of interconversion of the pyrimidine nucleotides. For instance, the activity of desoxycytidine deaminase disappears 16-18 h after irradiation, while the activity of the thymidylate-kinase systems is sharply inhibited [15]. In the light of these factors, pseudouridinuria is more likely to be the result of a block of the enzymes of biosynthesis of the pyrimidine nucleotides than the result of an increase in their biosynthesis. Consequently, acute irradiation is characterized by an increase in the excretion not only of the pyrimidine nucleoside of DNA – desoxycytidine [1], but also of the fifth nucleoside of RNA – pseudouridine. The relationship between these two phenomena and their importance in the pathogenesis of the radiation process are matters requiring further investigation.

SUMMARY

The object of study was the degree of excretion of 5-ribosyluracil (pseudouridine) in 8 healthy subjects and in 8 cancer patients before and after local x-ray irradiation. In healthy men the diurnal excretion of pseudouridine with urine is 56.0 mg on the average. In patients with lung and esophageal cancer the average amount of pseudouridine excretion with urine is close to normal, but there are large individual variations. Single local exposure to x-rays, in a dose of 100 R practically fails to influence the degree of pseudouridine excretion, while multiple local x-irradiation of cancer patients in a total dose of 1800 R leads to an increase in pseudouridine excretion with urine by 100% on the average.

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