EXCRETION OF PSEUDOURIDINE IN THE URINE OF TUMOR-BEARING RATS BEFORE AND AFTER WHOLE-BODY IRRADIATION

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The concentration of pseudouridine in the urine of healthy rats and of rats with transplanted sarcoma 45 is practically the same. Whole-body irradiation in a dose of 600 R caused pseudouridinuria in both groups of rats to an equal degree.

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We previously discovered an increase in the concentration of pseudouridine (PSU) in the urine of patients with lung cancer after therapeutic irradiation in a total dose of 2000 R [2]. However, considerable individual variations in PSU excretion by these patients and an increase in its content in the urine of patients with leukemias [3, 4] make special experimental investigations of this problem necessary.

In the present investigation the excretion of PSU in the urine of tumor-bearing rats was studied before and after irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar albino rats weighing 135-150 g. The rats were divided into two groups with 20 animals in each group. The 24-h excretion of PSU in their urine was determined for three days. The rats of group 1 (20 animals) then received whole-body external irradiation in a dose of 600 R (dose rate 150 R/min) on a 5 MeV linear accelerator, and their PSU excretion also was studied during the first three days after irradiation.

The rats (20) of group 2 were inculated with sarcoma 45 (10 intramuscularly and 10 intraperitoneally). Two weeks after inoculation, when the tumor mass was of considerable size, the 24-h excretion of PSU in the urine was again determined for 3 days. The tumor-bearing rats of both groups were then irradiated under the same conditions. Determinations of PSU before and after irradiation were carried out in urine collected from 20 and from 10 rats (in the groups of tumor-bearing animals), and also were calculated per animal.

During the experiment the rats were kept in metabolic cages with arrangements for collecting the urine separately.

The PSU content was determined by our suggested method for determining PSU and thymidine simultaneously in the urine.

The urine was alkalified with ammonia to pH 11.0, the precipitated phosphates were removed, and the liquid diluted with ammonia (pH 11.0) twice. A portion of this dilution (10 ml) was applied to a column with the anion-exchange resins Dowex 1×8, 200/400 mesh, in the formate cycle, measuring 1 × 10 cm, preliminarily washed with 0.1 N NH_4OH solution to pH above 10.0. After the urine had soaked into the column, 5 ml of 0.1 N NH_4OH was run through, and elution was then carried out with 0.1 N NH_4COOH solution (pH 7.0). The first 15 ml of eluate contained 5-ribosyluracil and thymidine. Numerous derivatives of the purines remained fixed on the column. The 15 ml of eluate thus obtained was evaporated in vacuo at a temperature of 18-20° inside the evaporating liquid. The residue was dissolved in a minimal volume of

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TABLE 1. Excretion of PSU in Urine of Healthy and Tumor-Bearing Rats before and after Whole-Body Irradiation in Dose of 600 R (in mg/24 h)

Animals	Before irradiation			After irradiation		
	1st day	2nd day	3rd day	1st day	2nd day	3rd day
Healthy	0.48	0.35	0.56	1.22	0.97	0.39
Tumor-bearing (tumor inoculated intramuscularly)	0.39	0.45	0.48	1.03	0.76	0.41
Tumor-bearing (tumor inoculated intraperitoneally)	0.51	0.73	0.49	1.44	0.67	0.32

distilled water and an aliquot part applied to Whatman 20 chromatographic paper. Chromatography was carried out in a system of isopropyl alcohol–formic acid–water (6:3:1) for 20-25 h. After drying, two clearly defined absorption stains were found in the ultrachemiscope with R_f values for uracil of 0.64 (5-ribosyluracil) and 1.08 (thymidine). The R_f values of these substances at the solvent front were 0.39 and 0.67 respectively. The stains were eluted with 3.5 ml 0.05 N HCl and optical density determined at 263 mμ for 5-ribosyluracil and at 267 mμ for thymadine. The content of these compounds in eluate of the stains were calculated from the coefficient of molar extinction, and from this value the content in the 24-h urine was determined.

EXPERIMENTAL RESULTS

The values obtained for the PSU content in the urine are given in Table 1.

No difference was found on comparing the level of excretion before irradiation in the healthy rats and rats with sarcoma 45. It is interesting to note that the PSU content in the urine of rats, expressed per kilogram body weight, was 3–4 times greater than in man [2]. A small increase in PSU content was found in animals with intraperioneal tumors. External whole-body irradiation in a dose of 600 R increased the excretion of PSU in the urine by 2-2.5 times on the first day after irradiation. On the second day this increase was smaller, and on the third day the PSU content was within normal limits.

A solid tumor (sarcoma 45) thus caused no increase in PSU excretion in the urine. External irradiation was equally effective in increasing PSU excretion both by healthy animals and by rats with tumors.

The fact that leukemias are accompanied by increased PSU excretion, while this is not observed in the presence of solid tumors, is evidence of specific disturbances in metabolism of transfer RNA in patients with leukemias. The view is held [5] that the turnover of transfer RNA in the leukocytes is faster in patients with leukemias. As we showed in previous experiments with C^{14}-orotic acid, the source of the increase in PSU in the urine after whole-body irradiation is increased breakdown of transfer RNA synthesized before irradiation [1].

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