AFRRI SR69-5
MARCH 1969

DEOXYCYTIDINURIA AND LYMPHOCYTOPENIA AS INDICATORS OF ABSORBED RADIATION DOSE IN RATS

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
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ACKNOWLEDGMENT

The authors gratefully acknowledge the help of T. A. Strike, Projects Director, and the technical assistance of R. H. Crutcher, E. L. Barron, M. E. Flynn, W. W. Wolfe and N. L. Fleming.

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FOREWORD
(Nontechnical summary)

The objectives of this investigation were (1) to evaluate excretion of deoxycytidine (CdR) in urine of rats as an indicator of absorbed dose of fast neutrons or mixed gamma-neutron radiations; and (2) to evaluate the radiation-induced depletion of circulating lymphocytes as related to the excretion of CdR.

Rats were exposed to radiation doses in the range of 22 to 780 rads and their urines were collected for analysis of CdR. Excretion of CdR was found to be a function of dose during the 4- to 12-hour interval after exposure. No significant differences in the dose-response curves for CdR excretion were observed between groups of rats exposed to fast neutrons and groups exposed to mixed gamma-neutron radiations.

As a first step in testing the hypothesis that destruction of lymphocytes by radiation is a contributor to deoxycytidinuria, a group of 10 rats was exposed to the same dose of x rays (50, 150, or 450 rads) on three successive days. The 4- to 12-hour postirradiation excretion of CdR was dose dependent after the initial exposure. After the second and third exposures when the numbers of circulating lymphocytes had been reduced, the excretion of CdR was not dose dependent, e.g., after an accumulated dose of 900 rads in 2 days, an additional 450 rads on the third day resulted in less excretion of CdR by irradiated rats than by unirradiated controls. The total CdR excretion after an accumulated dose of 150 to 1350 rads approximated that of an equal single dose. Calculations of the amount of CdR excretion per deleted circulating lymphocyte suggest that there are other radiosensitive cells that

contribute to deoxycytidinuria. However, when rats have been depleted of circulating lymphocytes by previous irradiation, an additional exposure to x rays does not elicit the deoxycytidinuria response.

ABSTRACT

The effect of doses of mixed gamma-neutron radiations or 14 MeV neutrons on the urinary excretion of deoxycytidine (CdR) was evaluated as a biodosimeter in rats. The rats were exposed to doses in the range from 22 to 780 rads. The excretion of CdR in the urine of irradiated rats during the 4- to 12-hour postirradiation interval was a function of dose over the range of 22 to 376 rads. This response was similar for the different types of radiations.

In a group of 10 rats exposed to the same dose of x rays (50, 150, or 450 rads) on three successive days, the 4- to 12-hour postirradiation excretion of CdR was dose dependent after the initial exposure but not after the second and third exposures. The total CdR excretion after an accumulated dose of 150 to 1350 rads approximated that of an equal single dose. The disappearance of circulating lymphocytes paralleled the urinary excretion of CdR. After lymphocytes had essentially disappeared following two 450-rad doses of x rays, a third dose of 450 rads induced less excretion of CdR by irradiated rats than by unirradiated control animals. Calculations of the amount of CdR excreted per deleted circulating lymphocyte suggest that there are other radiosensitive cells that contribute to deoxycytidinuria.

I. INTRODUCTION

Dose-dependent increases in urinary concentrations of pyrimidine deoxyribosides have been investigated in different species following x or gamma irradiation. Parižék et al.[12] were the first to report that during the first 24 hours after whole-body x irradiation the excretion of deoxycytidine (CdR) in rat urine increased with dose over a range of about 20 to 300 rads. Deanović et al.[3] reviewed several reports on increased concentration of metabolites in the urine of x irradiated mammals and suggested that CdR was a possible specific indicator of radiation injury. Later reports confirmed the gamma and x irradiation effect on CdR excretion in rats.[2,6,17]

Parižék et al.[12] hypothesized that x irradiation caused either increased release of CdR from DNA or decreased synthesis of DNA and a resulting accumulation of CdR in rat urine, but they did not suggest a tissue source of the DNA. Tereshchenko,[21] Guri et al.[6] and Drahovský et al.[5] reported that the spleen was a source of the CdR because preirradiation splenectomy decreased postirradiation deoxycytidinuria. It was suggested that the origin of CdR was DNA or polydeoxyribonucleotides released in the spleen, but the amount of excreted CdR exceeded that which could be obtained from splenic DNA.[6,21]

The number of circulating lymphocytes, one of the most radiosensitive animal cells, is reduced to 25 percent of normal in rats 4 hours after exposure to 100 R of x rays.[7] CdR excretion 4 to 12 hours postirradiation is a function of the dose, and thus it seemed possible that the excretion of CdR is related to the destruction of lymphocytes.

This paper reports the effect of mixed gamma-neutron radiation or 14 MeV neutron doses on the urinary excretion of CdR by rats. The relationship of deoxycytidinuria and lymphocytopenia was also evaluated in rats exposed to x rays.

II. MATERIALS AND METHODS

Male and female Sprague-Dawley rats* were acclimatized for 2 weeks before experimentation. The rats were fasted from 20 hours prior to irradiation until the end of the experiment, but water was continuously available.

Groups of ten rats were unilaterally exposed to 47, 94, 188, 348, 376, or 752 rads of mixed gamma-neutron radiations while comparable control groups were sham irradiated. The animals, weighing 201 to 321 g, were irradiated in individual Plexiglas boxes. In each dose group rats were placed so that their midlines were in an exposure field in which the tissue kerma, free-in-air, did not vary by more than 4 percent from the mean. Doses are reported as midline tissue doses. Approximately 60 percent of the tissue kerma, free-in-air, was attributed to gamma rays and 40 percent to neutrons. Exposures were made using a steady-state mode of operation of a TRIGA Mark-F reactor for 10 minutes. Details of the exposure array, reactor characteristics, and methods of dosimetry have been previously described.[4,14,18]

An additional experiment was conducted in which five groups of 10 male rats were exposed to 47-, 94-, 188-, 376-, or 780-rad doses of mixed gamma-neutron radiation attenuated by a 4-inch lead shield. The shield increased the neutron contribution to approximately 90 percent of the total tissue kerma, free-in-air.

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* Simonsen Laboratories, Inc., White Bear Lake, Minnesota

For 14 MeV neutron studies, groups of 6 male rats were exposed to radiation from a neutron generator.* The three lowest doses (22, 45, and 87 rads) were given at an average dose rate of 10 rads/minute and the other doses (164, 252, 334, 445, and 660 rads) were given at 16 to 20 rads/minute. The rats were exposed in a rotating circular Plexiglas array. Doses were determined by sulfur activation and with tissue-equivalent ionization chambers.

For lymphocyte studies, groups of 10 male Sprague-Dawley rats weighing 235 ± 2 g were exposed to the same dose of x rays (50, 150, or 450 rads) on three successive days. Radiation factors for the x-ray generator were: 250 kVp, 30 mA, HVL 1.90 mm Cu, filter 1.2 mm Be plus 0.95 mm Cu, 19 rads/minute, and target to animal distance of 100 cm. Prior to each exposure, blood samples were obtained from the tail of the restrained, unanesthetized rats. White blood cells were counted by a Coulter Model B electronic cell counter.† The absolute number of lymphocytes per mm^3 was calculated from the total number of white blood cells and the differential counts. It was assumed that the total blood volume in the rat was 6.7 cm^3/100 g body weight.[1]

All rats were placed in individual metabolism cages as soon as they could be retrieved from the exposure room. At 4, 8, 12, 24, and 48 hours postirradiation, urine was collected, stored at -20°C, and later analyzed for CdR.

For CdR analysis, a 2-ml sample of urine was diluted fivefold with water and passed through a column (1 x 2.5 cm) of cation exchange resin, AG-50W-X8 (100 mesh), to absorb CdR.[8] The samples were eluted with ammonium hydroxide, dried, extracted

* Model 9505, Texas Nuclear Corporation, Austin, Texas
† Coulter Electronics, Hialeah, Florida

with alcohol, dried again, dissolved in water, and mixed with cysteine-sulfuric acid to develop the colored solution. The absorbancy of the colored compound formed from CdR and other Dische positive substances, which developed adequately after 2 hours at 25°C, was determined at 490 nm with a spectrophotometer.[19] The concentration of CdR was calculated using calibration curves obtained from similar analyses of standard solutions of CdR.

III. RESULTS

The excretion of CdR in the urine of irradiated male rats during the period 4 to 12 hours postirradiation increased as a function of the dose over the range from 47 to 376 rads of mixed gamma-neutron (3:2 or 1:10) radiations and over the dose range from 22 to 334 rads of 14 MeV neutrons (Figures 1 and 2). The CdR excretion during the 0- to 4-, 12- to 24-, and 24- to 48-hour intervals was not dose dependent.

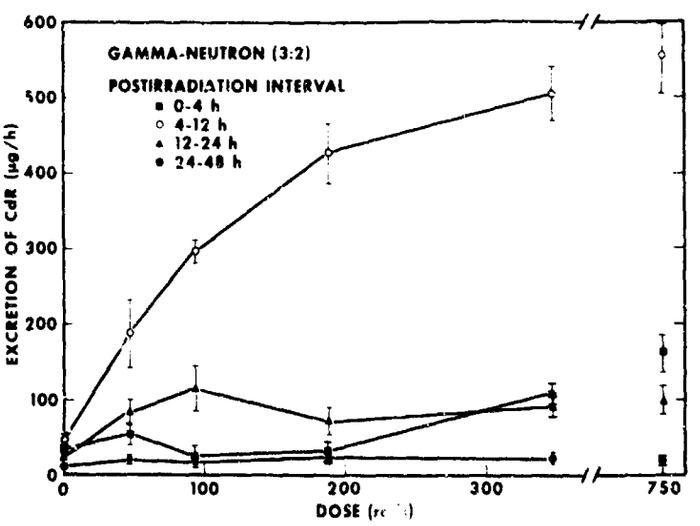

Figure 1. Postirradiation excretion of deoxycytidine in male rats exposed to mixed gamma-neutron radiation. The data points represent mean values with the associated standard error.

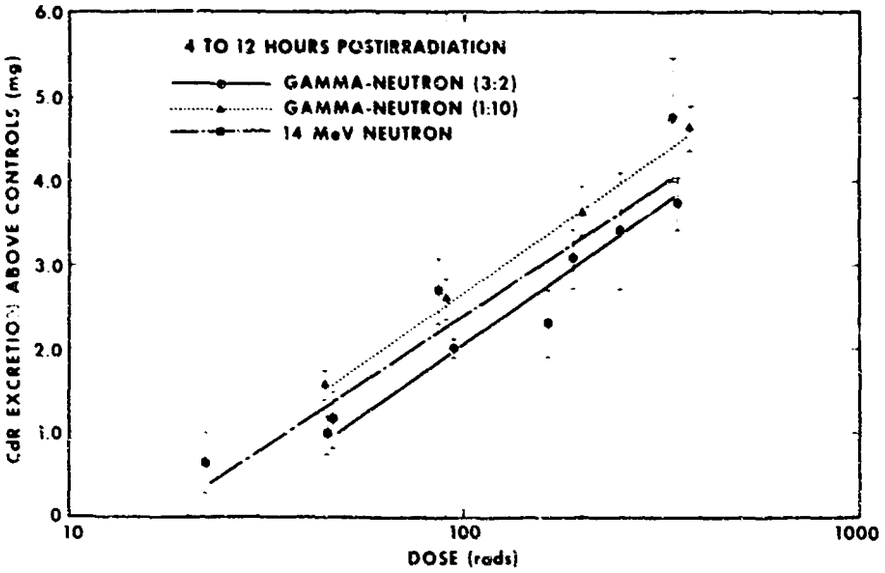

Figure 2. Postirradiation deoxycytidinuria in male rats exposed to mixed gamma-neutron or 14 MeV neutron radiation. The data points represent mean values with the associated standard error.

To compare the postirradiation deoxycytidinuria of male and female rats, the excretion was calculated as micrograms CdR per gram of body weight to compensate for differences in body weights. After rats were exposed to mixed gamma-neutron (3:2) radiations, the postirradiation excretion of CdR per gram of body weight in female rats was not significantly less than that of male rats.

Table I presents the results obtained by exposing each group of rats to a similar dose of x rays (50, 150, or 450 rads) on three successive days. Prior to the x-ray exposure there was very little variation in the number of lymphocytes per mm^3 of peripheral blood among the groups of rats. The 4- to 12-hour postirradiation excretion of CdR was dose dependent after the first x-ray exposure, but on days 2 and 3 when the lymphocyte count had been markedly reduced, the excretion of CdR was not dose dependent. In fact, after the second exposure, the number of lymphocytes was

a more reliable indicator of absorbed dose than was CdR excretion. After a third exposure to a dose of 450 rads, when the lymphocytes had been almost totally depleted, the excretion of CdR was even less than that of unirradiated controls. The total CdR excretion after an accumulated dose approximated that of an equal single dose (Table I). (Similar results were obtained in preliminary studies using mixed gamma-neutron radiations.)

Table I. Lymphocytopenia and Deoxycytidinuria in Rats During Three Daily Exposures to Radiations (x rays)

Daily dose (rads)	Lymphocytes per mm^3 before each exposure*			CdR excretion 4 to 12 hours after each exposure* (μg)			
	1	2	3	1	2	3	Total
0*	8959 ± 474	7826 ± 633	7058 ± 681	304 ± 40	248 ± 32	248 ± 48	800
50	8654 ± 735	3556 ± 200	1884 ± 376	1191 ± 208	690 ± 136	344 ± 128	2225
150	8982 ± 560	1380 ± 105	391 ± 40	2780 ± 224	1000 ± 136	248 ± 32	4028
450	8242 ± 752	383 ± 120	94 ± 32	3870 ± 336	524 ± 44	133 ± 16	4527

* Mean values with the associated standard error
* Unirradiated controls

To determine if the excretion of CdR was constant per destroyed lymphocyte, the number of circulating lymphocytes deleted per rat during the 24 hours after the first exposure was divided by the CdR excreted during maximal CdR excretion (4 to 12 hours postirradiation). These calculations showed that the doses of 50, 150, and 450 rads induced the excretion of 11, 21, and 29 picograms of CdR per deleted circulating lymphocyte, respectively. The lack of a constant value and the calculation that these values are greater than the cell content indicate that other radiosensitive cells contribute to the postirradiation deoxycytidinuria.

IV. DISCUSSION

To be a useful biodosimeter, a biochemical effect resulting from exposure to ionizing radiation should be dose dependent over the range when alteration of mortality by therapeutic measures is most promising and an accurate assessment of radiation injury is important. Parižék et al.[12] first reported that the urinary excretion of CdR in rats was a sensitive indicator of exposure to low doses of x rays. Their results indicated that as the dose increased from 0 to 300 rads, the CdR excretion for the first 24 hours postirradiation increased from 1 to 7 mg. However, CdR excretion following 300- and 600-rad doses were not significantly different from each other, indicating the response was not dose dependent beyond 300 rads. Skurikhina and Tereshchenko[17] reported that the excretion of CdR in rats exposed to gamma radiations in the dose range of 0 to 10,000 rads increased from 0.2 to 7 mg during the first 24 hours postirradiation; however, this dose dependency above 300 rads of ionizing radiation has not been confirmed. Guri et al.[6] reported that CdR excretion in female rats exposed to x irradiation increased from 0 to 400 μg CdR over base-line values for the first 24 hours after the rats were given doses in the range of 0 to 400 R. This amount of CdR (0.4 mg) excreted by the rats exposed to a dose of about 400 rads was considerably less than that reported by Chen et al.[2] (5 mg), Parižék et al.[12] (6 mg), and the value currently reported (4 mg) for males or females. In the present report, the time of maximal rate of excretion (4-12 hours) of CdR includes the interval reported by Guri et al.[6] (4-8 hours), but it is somewhat earlier than the interval reported by Chen et al.[2] (9-18 hours).

In the present study, the dose response curves (Figure 2) for different qualities of radiation are the same within their statistical uncertainty. Thus, the relative biological effectiveness of the mixed gamma-neutrons and the 14 MeV neutrons for this end point is one.

The number of lymphocytes per mm^3 of peripheral blood may be regarded as providing an approximate measure of the whole lymphocyte population in the intra- and extra-vascular circulation[7]. In the current report, it is suggested that the depletion of these lymphocytes is related to the postirradiation excretion of CdR for the following reasons: (1) the major depletion occurs within 4 hours[7] and the maximal CdR excretion within 4-12 hours; (2) there is no increased postirradiation excretion of CdR above unirradiated controls in irradiated rats having essentially no circulating lymphocytes; and (3) both CdR excretion and destruction of lymphocytes[15] respond similarly in the same dose range. However, if only circulating lymphocytes were involved, the excretion of CdR per deleted lymphocyte would have been relatively constant, instead of the progressive increase with dose found in this study. This suggests that there are other radiosensitive cells that contribute to deoxycytidinuria.

Attention has been centered on the spleen as a source of CdR in postirradiation deoxycytidinuria. In irradiated rats, Drahovský et al.[5] and Guri et al.[6] reported that the spleen played the principal role in postirradiation deoxycytidinuria. However, the amount of CdR excreted was greater than that which could have been formed by disintegration of spleen DNA.[6,21] It was therefore assumed that the spleen takes part in the development of postirradiation disturbance of DNA metabolism in other tissues or organs and in some way releases CdR.[21] Neff and Cassen[11] postulated

that some organs recognized injured lymphocytes and remove them from circulation. Since the spleen is considered to be the principal organ for destruction of blood cells,[9] the role of the spleen could be to recognize and catabolize circulating lymphocytes injured by radiation. Free deoxypolynucleotide of DNA accumulated in rat spleen, thymus, and bone marrow about 4 to 6 hours postirradiation.[13,16] Swingle and Cole[20] reported that the polydeoxyribonucleotides were formed initially from DNA in lymphoid tissues of irradiated rats by action of DNase. Active DNases in blood[10] might account for enzymatic hydrolysis of DNA and polydeoxyribonucleotides yielding CdR which accumulates in blood and urine.

V. SUMMARY

The urinary excretion of deoxycytidine by irradiated rats during the 4- to 12-hour postirradiation interval was a function of dose over the range of 22 to 376 rads. There were no apparent differences in excretion of CdR in rats exposed to mixed gamma-neutron radiations (gamma-neutron ratio of 3:2 or 1:10) or to 14 MeV neutrons in the same dose range.

When a group of rats was exposed to a similar dose of x rays (50, 150, or 450 rads) on three successive days, the 4- to 12-hour postirradiation excretion of deoxycytidine was dose dependent only after the initial exposure. After the circulating lymphocytes had been depleted by an accumulated dose of 900 rads in 2 days, a dose of 450 rads on the third day induced less excretion of deoxycytidine in irradiated rats than in unirradiated controls. Calculations of the amount of CdR excreted per deleted lymphocyte indicate that there are other radiosensitive cells that contribute to deoxycytidinuria.

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1 ORIGINATING ACTIVITY (Corporate author) Armed Forces Radiobiology Research Institute Defense Atomic Support Agency Bethesda, Maryland 20014	2a REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b GROUP N/A

3. REPORT TITLE

DEOXYCYTIDINURIA AND LYMPHOCYTOPENIA AS INDICATORS OF ABSORBED RADIATION DOSE IN RATS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (Last name, first name, initial)

Skidmore, W. D. and McHale, C. G.

6. REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
March 1969	19	21

8a. CONTRACT OR GRANT NO b. PROJECT NO. c. R MD 3 9013 d.	9a ORIGINATOR'S REPORT NUMBER(S) AFRRI SR69-5 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. AVAILABILITY/LIMITATION NOTICES

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