DEOXYCYTIDINE LEVELS IN URINE OF X-IRRADIATED RATS

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A number of investigators have demonstrated that irradiation of animals to lethal and sublethal doses of ionizing radiation impaired the metabolism of ribonucleic and especially deoxyribonucleic acids. An experimental approach to the problem is to determine the existence of amounts of catabolites of deoxyribonucleic acids or of their intermediary products in the urinary excreta of irradiated animals.

Parizek et al reported that the urine of irradiated animals (up to 600 r) contained substances that gave a positive reaction for desoxyribose. Later Kosyak et al reported increased excretion of desoxyribosides by irradiated rats (400 to 800 r) and by dogs (500 r).

In these earlier studies the cysteine-sulfuric acid reaction described by Dische was used. The reaction measures all substances containing deoxyribose. Similar colored reactions are produced, for instance, by 2-deoxyribose, DNA, and some nucleotides.

Of primary interest in the study reported here is deoxycytidine, included in the substances containing desoxyribose. Deoxycytidine, or cytosine desoxyribose as it is often called, is one of the major pyrimidine nucleoside components of deoxyribonucleic acid.

It has been possible, both by the investigators mentioned above and by our group, to use chromatographic techniques as a means of separating substances

containing deoxyribose and to measure specifically amounts of deoxycytidine. Our group has also used a microbiological assay method for quantitative determination of deoxycytidine.

A program was then undertaken to study the ~~urinary~~ excretion of deoxycytidine in the urine of ~~irradiated~~ rats exposed to various doses of x-radiation.

METHODS Wistar rats, weighing 170-200 grams, were placed in pairs in metabolic cages. For the irradiation exposure rats were placed two at a time in a lucite cage and exposed to doses (200r, 500r, 800r doses were used) of whole body irradiation delivered by an x-ray unit operated at 250 kvcp having a half-value layer of 1.80 mm Cu. The dose rate was measured with a 100r Victoreen ionization chamber placed in paraffin rat phantom to simulate actual experimental conditions. To insure uniform irradiation of the rats, the cage was rotated during the exposure. Control rats were pham-irradiated.

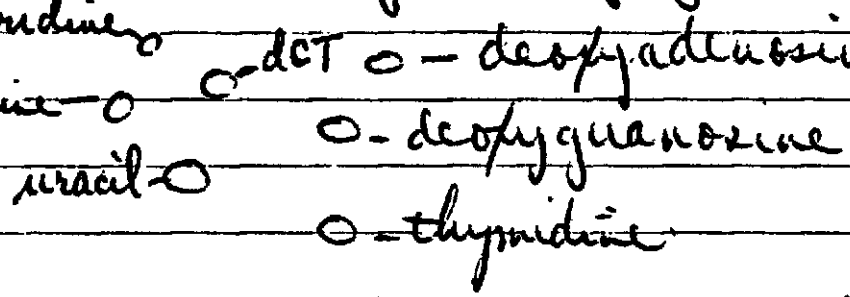
Urine samples were collected for 24 hours pre- and for 0-24, 24-48, and 48-72 hours post irradiation. Specimens were collected in toluene and were frozen immediately afterward until they were analyzed. Specimens from two rats receiving the same radiation dose were pooled. The urine was acidified to pH 1 with an HCl activated charcoal 2% aqueous suspension. Nucleic acid derivatives including deoxycytidine

were adsorbed on the charcoal. The charcoal suspension was centrifuged and the clear supernatant was removed and discarded. Ten per cent aqueous pyridine was added to the charcoal and the suspension was allowed to incubate at 37° in a water bath with a mechanical shaker for 3 hours. The suspension was again centrifuged and the pyridine, now containing nucleic acid derivatives, was withdrawn and placed in a small evaporating dish. The pyridine was removed in vacuo at room temperature. The residue was taken up in 1 ml distilled water and stored in a freezer prior to chromatography.

Both one and two dimensional chromatograms were prepared using 10, 25 and 50 µl aliquots of concentrated nucleic acid residue. The two-dimensional chromatograms were necessary because hippuric acid and para-hydroxy hippuric acid, present with nucleic acid derivatives in the final residue, interfered with separation on one-dimensional chromatograms.

The finished chromatograms were examined under ultraviolet light (short wavelength). Nucleic acid derivatives appeared as dark (absorbing) spots. Permanent records of the chromatograms were made by placing each over a sheet of Kodagraph contact paper, exposing to ultraviolet light for seconds, and developing with Kodak developer and fixer.

Figure 1 shows a typical chromatogram record of some ultraviolet absorbing substances to indicate the location of deoxycytidine.



The substance at this location (found in rat urine after extraction and concentration) was proved to be deoxycytidine by co-chromatography and rechromatography with chromatographically pure dCT and by elution of the substance from the paper and measurement of the absorption spectrum.

Butanol acetic acid

Ammonium Sulfate

Thymine not used by rats in nucleic acid formation.

A microbiological assay using Lactobacillus leichmanii has been used for some of the work in quantitating the dCT. The assay is based on the fact that deoxyribosides can replace the requirement of the test organism for vitamin B_{12}. Following the chromatographic procedures mentioned above, strips of chromatograms were placed on nutrient agar containing the constituents of the medium used for assay of B_{12} and seeded with the organism. After incubation growth zones corresponding to DOC were noted. A drawback to the use of the microbiological assay appeared when an amount of dCT greater than 2-3 µg was present in the chromatogram. The logarithmic growth response versus dCT concentration was not observed with a concentration of dCT over 3 µg with the numbers of specimens involved and with the high dCT levels noted, rather than prepare additional chromatograms containing smaller amounts of nucleic acid concentrate for microbiological assay, we elected to use ultraviolet spectrophotometric measurement of dCT for quantitative determination. Known amounts of dCT were spotted on paper,

RESULTS

A urinary deoxycytidine mean level of 29 microgram/rat (range of) was found in the control (non-irradiated) rats. Deoxycytidine was recovered from the urine of x-riradiated rats, with peak output occurring during the first twelve hours post-irradiation (mean levels of 244 µg/rat, 1000 µg/rat and 827 µg/rat for 200, 500 and 800 r radiation doses, respectively). The deoxycytidine levels in urine remained high for the 12-24 hour period post-irradiation, dropped sharply during 24-48 hour period, and by 48-72 hours had almost returned to pre-irradiation control values. This data is shown in Figure 1, with the 0-12 and 12-24 hour values combined for a 0-24 hour value. Both the mean levels and ranges are given. Figure 2 gives the mean level (and range) of deoxycytidine in the urine of rats before and after irradiation with 0, 200, 500 and 800 roentgens

800-R 0-24 1611 (1056-2660)
236 (66-450)
114 (10-280)

0-24 hr post 500R 484 (248-585)

500R 1755 (630-3050)

800R 1611 (1056-2660)

DISCUSSION

~~In summary,~~ The results do show marked excretion of dCT in the urine of rats after irradiation. Fractionation of the nucleic acid derivatives on paper chromatograms was necessary to demonstrate the excretion of dCT by the rats. ~~Of interest~~ dCT fulfills many of the characteristics desirable of a biological dosimeter. Peak values occur soon after irradiation exposure (0-7.4 hrs, although high levels were noted in 0-12 hr period). Minimal amounts of dCT are present in normal rats. ~~Other biological dosimeters suffer in that fairly high ratios and fluctuating values are measured in normal rats, so that it becomes difficult to determine precisely early increased changes post irradiation.~~ Parzek has indicated that other stresses to rats, as trauma, burns, injection of formaldehyde did not produce in the urine increased amounts of basiche positive substances. Based on the preliminary results, no quantitative dose-response relationships was found, although the urine dCT levels at 500 r and 800 r doses were considerably higher than levels observed in rats exposed to 200 r. Of interest is the process by which marked increases of dCT take place. ~~Some remarks on this point will be made in the following pages.~~

no # It may be that irradiation inhibits the synthesis of deoxyribonucleic acid at the stage of this nucleoside. It may also be possible that as a result of the irradiation, the formation of deoxycytidine from deoxyribonucleic acids is increased.