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Note

Separation of deoxycytidine from urine by ion-exchange chromatography

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The level of deoxycytidine (CdR) in rat urine was first suggested as a biochemical indicator of radiation injury by Parizek *et al.*[1]. Their work has been confirmed by several authors including Guri *et al.*[2].

There is species variation in the normal excretion of CdR. Rats excrete between 70 and 100 μg in 24 h (ref. 2). The mouse and human excrete less than 10 μg (40 nmoles) (ref. 3). There is a need for the development of a sensitive method of detecting CdR that will allow radiation/excretion studies to be carried out with samples from mice and human patients.

A microbiological assay reported elsewhere has been used successfully for CdR determination in human and animal urine[4]. The limit of detection has been estimated as 0.3 μg (1.3 nmoles) CdR in a 24-h urine specimen. The disadvantage of this method is that the assay time can be as long as 2.5 days. An alternative method employs a colorimetric technique based on the malonaldehydethiobarbituric acid reaction for the estimation of deoxy sugars[5]. This method is not as sensitive as the microbiological assay, it relies on the effective separation of CdR from other urinary deoxy sugar compounds and it requires the previous hydrogenation of thymidine and deoxyuridine.

Cohn[6,7] investigated the separation of the major nucleosides from urine by ion-exchange chromatography using either cation or anion resins but he did not study the trace nucleosides such as deoxycytidine.

Recently Gerber[8] reported a technique for concentrating deoxycytidine from urine by ion-exchange chromatography prior to purification by paper chromatography and microbiological determination.

An ion-exchange system for the separation of CdR from other urinary UV-absorbing substances, together with its subsequent estimation in the same process, is described in this note. The aim of the work is faster analysis with appropriate sensitivity. The analysis time with human urine is about 12 h, with animal urine 5 h and the limit of measurement was approximately 10 nmoles in 24-h urine. The method was applied to rat, mouse and human urine.

MATERIALS AND METHODS

Purine and pyrimidine derivatives were obtained from Sigma London Chemical Co. (Kingston-upon-Thames, Great Britain); [^{14}C]CdR was obtained from the Radiochemical Centre (Amersham, Great Britain).

Animals were housed in metabolism cages and 24-h urine was collected using toluene or phenol as a preservative.

Partial purification and concentration

Filtered urine at pH 9.5 was applied to a column of AG 2-X8 (OH^-) anion-exchange resin. The resin was washed with 0.02 *N* NH_4OH. The CdR containing effluent was acidified and applied to a column of Zeo-Karb 225 (H^+) cation-exchange resin. The resin was washed with 0.4 *N* HCl. CdR was eluted with 1 *N* NH_4OH. The eluate was evaporated to dryness by rotary evaporation and dissolved in a small volume of acid solution. CdR was resolved by the following system. A scaled-up version of this procedure was used for human urine.

Column

A column (0.6×125 cm) of Chromobeads A (Na^+) (Technicon, Tarrytown, N.Y., U.S.A.) (>600 mesh) was used at 75°.

Elution

Gradient elution was employed. The gradient was produced by a two-chambered Technicon Autograd. The mixing chamber contained, initially, 150 ml of 0.3 *M*-citric acid–sodium citrate buffer pH 5.0. The second chamber contained 150 ml of 1.0 *M*-citric acid–sodium citrate pH 5.0. A non-linear increasing Na^+ concentration gradient at pH 5.0 was produced. The flow-rate was 0.38 ml/min. The pressure was about 200 p.s.i.

Detection

The effluent was monitored by passing it through a flow cell (1 cm path length) in a Uvichem H1600 spectrophotometer (Hilger and Watts). The wavelength was 280 nm. Either of two linear absorbance scales was used, 0–1 or 0–0.2. The spectrophotometer was linked to a pen recorder.

Deoxycytidine was quantitated by estimation of the peak area ($h \times w$); h is the maximum peak height and w is the peak width (millilitres) at half the maximum peak height[9,10].

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram produced with a mixture of some of the principal urinary purine and pyrimidine derivatives and deoxycytidine. The response factor for CdR (nmoles CdR/($h \times w$)) was 132. The CdR loss on this column, as determined with [^{14}C]CdR, was found to be negligible.

The minimum measurable amount of CdR on the 0–0.2-scale was about 5 nmoles.

Rat urine

Fig. 2 shows a typical chromatogram with the CdR peak centre at 66 ml. The overall mean recovery as determined with [^{14}C]CdR was 68%, the major loss occurring during rotary evaporation. The mean excretion was found to be 78 nmoles or 17.8 μg/24 h.

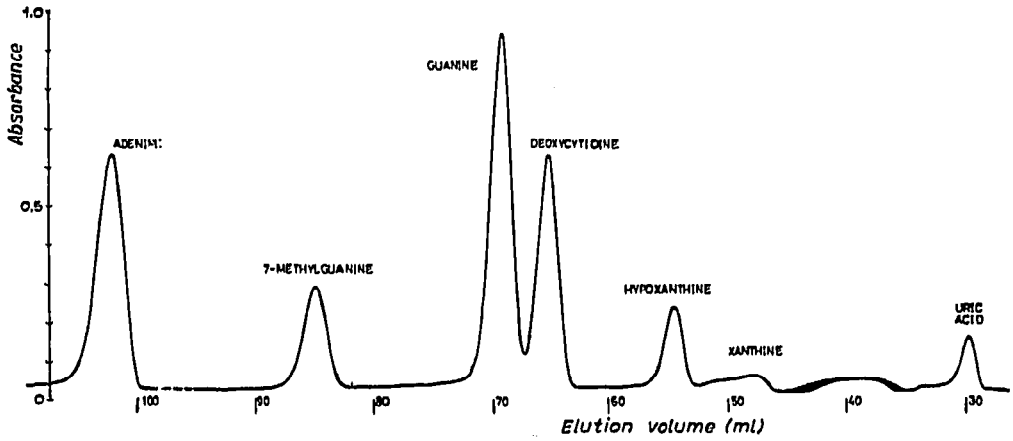

Fig. 1. Separation of some of the principal urinary purine and pyrimidine derivatives and deoxycytidine. Column, Technicon Chromobeads A (Na^+), 75°; elution, gradient (0.3 $M \rightarrow 1$ M) citric acid–sodium citrate buffer, pH 5.0; flow-rate, 0.38 ml/min; wavelength, 280 nm; uric acid, 0.04 μmoles; xanthine, 0.14 μmoles; hypoxanthine, 0.37 μmoles; deoxycytidine, 0.16 μmoles; guanine, 0.35 μmoles; 7-methylguanine, 0.15 μmoles; adenine, 0.46 μmoles.

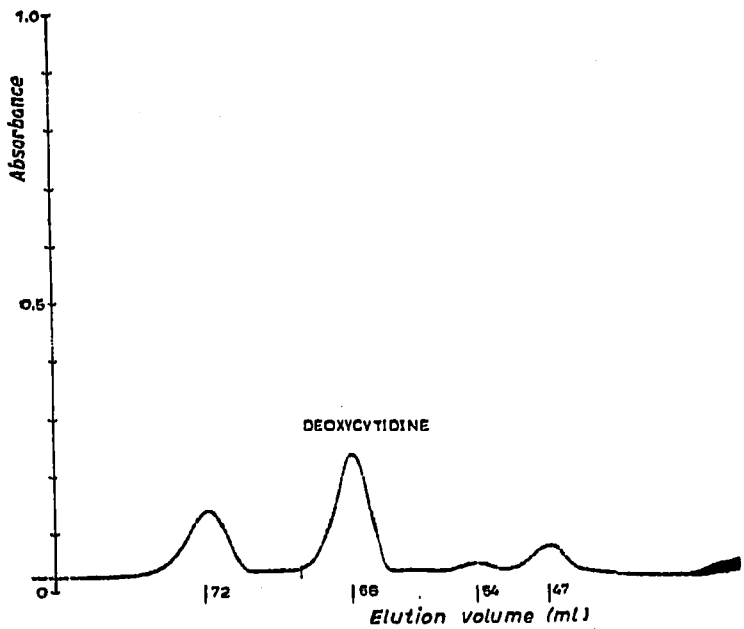

Fig. 2. Separation of CdR from rat urine. CdR peak is prominent at 66 ml. The other peaks were tentatively identified as xanthine (peak at 47 ml), hypoxanthine (peak at 54 ml) and guanine (peak at 72 ml).

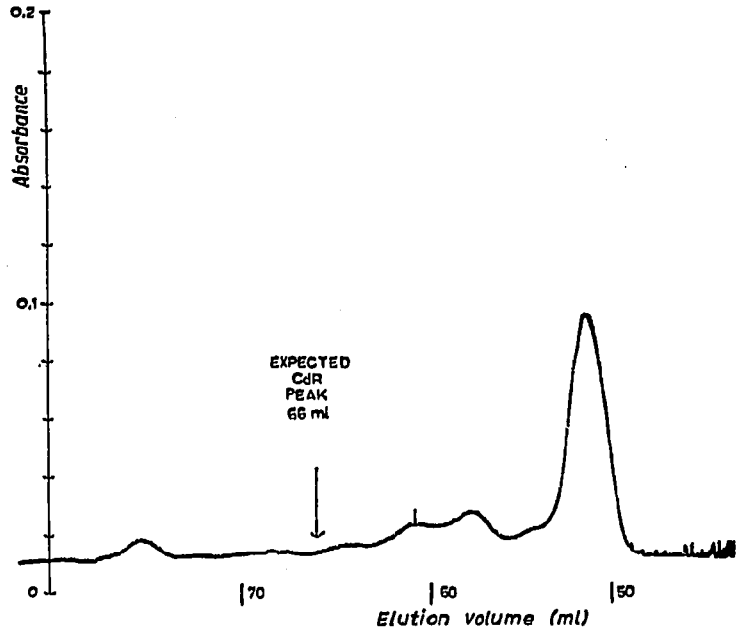

Fig. 3. Separation of CdR from mouse urine.

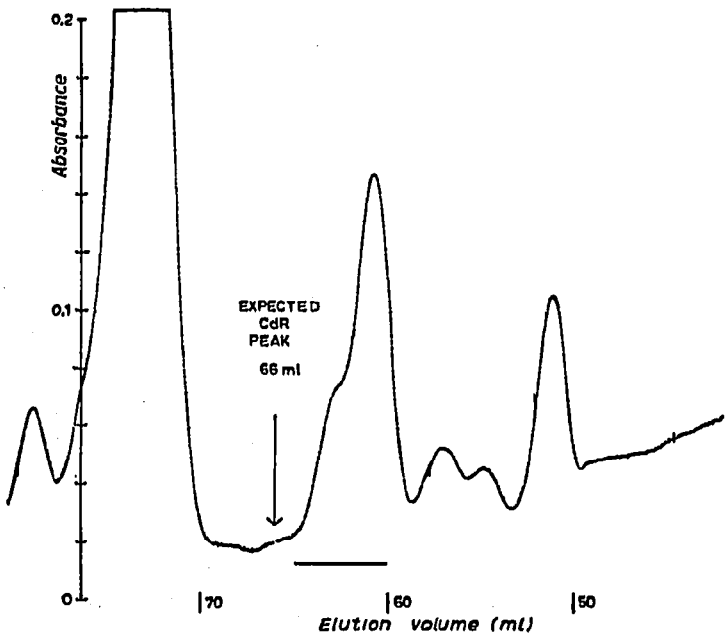

Fig. 4. Separation of CdR from human urine from a female patient.

Mouse urine

Fig. 3 shows a typical chromatogram derived from 24-h mouse urine. The overall loss was the same as with rat urine. CdR is undetectable. The CdR level in T.O.-strain mouse urine, taking into consideration the loss during processing, must be below the 10 nmole (2 μg) level.

Human urine

Fig. 4 shows a typical chromatogram derived from human urine. The overall mean recovery was 64%, the major loss occurring during rotary evaporation. CdR is not measurable. The CdR level in the human samples investigated must be below 10 nmoles (2 μg) in 24 h.

The data on normal values of CdR in human urine are variable. This seems to be related to differences in methodology. Silberstein *et al.*[11], using the colorimetric technique, reported that the normal range was 2–10 μg/24 h. Dienstbier *et al.*[3], using the microbiological technique, found the normal range from undetectable to 1.5 μg/24 h. We found CdR to be undetectable in 24-h human urine and below the 2-μg level.

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