THE EXCRETION OF $^{14}$C-HYPOXANTHINE AND ITS METABOLITES IN RATS FOLLOWING ADMINISTRATION OF URICOSTATIC DRUGS

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INTRODUCTION

In vivo xanthine oxidase inhibition results in a reduction of uric acid (and allantoin) in urine and in an increase in the urinary excretion of hypoxanthine and xanthine as described by Elion$^1$ for allopurinol. The dose-dependence of the excreted amounts of hypoxanthine and xanthine is a relevant consideration when using these parameters to measure the action of uricostatics in the rat. The purpose of our studies was to obtain a simple and rapid method to detect the uricostatic quality of hypouricemic compounds.

METHODS

Male Wistar rats (company's own breeding) with a body weight of 120 – 150 g were used. The last 16 hours preceding the experiment the animals were deprived of food but drinking water remained unlimited. Two rats of the same body weight were placed in one metabolism cage. All animals were taken from the same population (identical age and any other conditions). Each assay was based on the evaluation of the urine samples taken from two or three metabolism cages. The experiment started with the oral administration of the drug in starch gel; the control rats received the vehicle alone. One hour later the animals were given 50 ml tap water/kg b.w. by stomach tube; 40 μCi/kg b.w. $^{14}$C-hypoxanthine were simultaneously injected intravenously. Immediately thereafter urine collection was started and samples corresponding to the 1st and 2nd hours were separated. The total radioactivity was measured in $1 - 3 \mu l$ of the urine fraction by liquid scintillation. The distribution of the radioactivity was determined.
following separation by high pressure liquid chromatography. Using Lichrosorb RP 18 on a 250 × 4 mm column, 50 μl of urine containing 5 - 20 nCi radioactivity were sampled and eluted at room temperature with either 0.01 M ethanolamine phosphate at pH 3.9, or with 0.01 M potassium dihydrogen phosphate at pH 4.0. The flow was 2.0 ml/min. Radioactivity was detected with a solid scintillator (Berthold), the obtained peaks were recorded and integrated for three determinations of each sample. The retention times were 1.6 min for allantoin, 4.3 min for uric acid, 5.3 min for hypoxanthine and 6.3 min for xanthine. The results presented are the mean values of a total of two to five experiments.

RESULTS

Allantoin and uric acid plus traces of hypoxanthine and/or xanthine were excreted in the urine of control groups of rats which had received radioactivity in the form of hypoxanthine. The reproducibility of the data and the variation between samples of corresponding cages are demonstrated in Table 1. The form in which radioactivity was excreted agrees with earlier findings of Greger who studied the renal excretion of allantoin and urate in rats. The biological and analytical deviation of the mean values in Table 1 also applies for the results in Tables 2 - 4. The sum of the radioactive activity found in the compounds detected in the urine represents 95 % of the total excreted radioactivity.

42 % of the administered 14C-activity was eliminated in the urine within 2 hours. Table 2 demonstrates the effect of different doses of allopurinol on the elimination and distribution of 14C-derived from hypoxanthine.