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# Urinary chemistry of the normal Sprague-Dawley rat

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**Summary.** On the basis of metabolic in vivo studies using the adult male Sprague-Dawley rat, we present a comprehensive summary of urine species measurements conducted in our laboratory over the last several years, as well as those present in the literature.

**Key words:** Enzymuria – Oxalate – Sprague-Dawley rat – Urinanalysis

Establishment of a valid in vitro rat model for urolithiasis, nephrotoxicity, or drug toxicity is dependent upon the reproducibility of the experimental criteria and resulting data. In determining the degree of damage incurred, urine analysis of renal metabolites and filtrates is often used as an indirect or corroborative measure of the resulting injury. An investigator's primary concern, in this regard, must be to establish reliable control and virgule or normal basal values for interpretation of the experimental levels. In the current literature no comprehensive review exists of urinary data in rat models alone. Although the hematologic values are extensive, the reference sheets available from rat breeders offer few measures of urine chemistries. Furthermore, evaluation of existing research indicates that urine data are based upon comparatively small numbers of rats, and frequently the reference values are different. Often, urine collections are made at 8, 12, or 16 h intervals, ignoring the importance of circadian rhythms and the nocturnal habit of the rat. In other reported experiments, collections are made on groups of animals housed within one metabolic cage, thus pooling the urines of individual animals. Animals are or are not restricted from dietary intake before collections depending on the experiment. After examining the more comprehensive reviews of urinary data, were frequently found that the strain or sex of the rat was not cited, or that weanling rats were used for these reference values. In some of these reviews, the source of the original data was unclear, often lacking reference to sample numbers and methods of analysis. Furthermore, many references on urinary enzmyes fail to define their unit definitions or substrates used in analysis. In our experience, analyses can vary greatly from animal to animal, litter to litter, and from season to season. Therefore, on the basis of numerous and expanded studies using the male Sprague-Dawley rat conducted under our laboratory conditions, we have compiled the results of our urinalyses and compared them with those in the literature [1–9].

## Materials and methods

### *Housing*

Two hundred and sixty-three male Sprague-Dawley rats weighing 250–300 g were obtained from Charles Rivers Laboratories (Wilmington, Mass.). Animals were obtained from different litters at various times of the year. The data presented are a compilation from all animals. After a 10-day quarantine period following shipping, rats were weighed, randomly divided and acclimatized to housing in metabolic cages (Nalge Company) for 4 days before the onset of any experiment. Rats were allowed free access to deionized water and ground Purina Rat Chow 5001, provided as a mush. All food came from the same milling date and was maintained at 4 °C until use. Climate-controlled animal rooms were maintained on a 12-h light–dark schedule.

### *Urine collections*

Twenty-four urine collections were made in acid-washed cages in one of two ways: either on thymol or on vessels jacketed by wet-ice and maintained at 4 °C. Analysis of the two methods revealed no difference in the effect on urinary pH or microbial contamination. At the end of all collections, urine volumes were determined by volumetric pipets and pH was measured before any further analysis was conducted.

### *Urinanalysis*

Thymol-collected urines were treated with 6 M HCl, and shaken for 45 min until the pH reached 1–2 to assure dissolution of any urinary crystals. Acidified urines were analyzed for phosphorus [10], sulfate [11], ammonia [12], and oxalic acid [13, 14]. Potassium and sodium

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**Table 1.** Urinary calcium, magnesium, sodium and potassium, sulfate, phosphate, and ammonia

| Source | $Ca^{2+}$ | $Mg^{2+}$ | $Na^{+}$ | $K^{+}$ | $SO_4$ | $PO_4$ | $NH_4$ |
|---|---|---|---|---|---|---|---|
| UF | | | | | | | |
| mmol/l | 3.39 ± 2.14 | 21.4 ± 5.7 | 149 ± 60 | 267 ± 99 | 29.3 ± 9.3 | 35.8 ± 11.8 | 22.9 ± 7.1 |
| | (0.42 – 10.02) | (7.2 – 37.6) | (45 – 250) | (120 – 475) | (9.4 – 51.1) | (11.7 – 63.4) | (10.4 – 56.2) |
| | *n* = 263 | *n* = 204 | *n* = 204 | *n* = 204 | *n* = 204 | *n* = 253 | *n* = 204 |
| mg/kg per day | 12.9 ± 8.7 | 33.5 ± 10.0 | 241 ± 101 | 806 ± 163 | 245 ± 129 | 86.7 ± 34.1 | 27.4 ± 7.7 |
| | (4.6 – 30.9) | (23.6 – 50.0) | (88 – 307) | (290 – 1242) | (73 – 360) | (54 – 136) | (15.4 – 39.0) |
| Literature | | | | | | | |
| mmol/l | 12.0 ± 0.8 [1] | | 147 ± 2.7 [1] | (367 – 477) [2] | | | (13 – 29) [3] |
| | | | | 5.8 ± 0.11 [1] | | | |
| mg/kg per day | 3.0 – 9.0 [1] | 0.2 – 1.9 [1] | 90 – 110 [1] | 50 – 60 [1] | | 20 – 40 [1] | |

Values are the mean ± SD
*UF*, University of Florida; *n*, sample number
[*1*], Mitruka and Rawnsley (1977); [*2*], Loeb and Quimby (1989); [*3*], Van-Liew et al. (1978)

**Table 2.** Urinary oxalic and citric acid

| Source | Oxalic acid | | Citric acid |
|---|---|---|---|
| | Colorimetric | Enzymatic | |
| UF | | | |
| mmol/l | 1.05 ± 0.41 | 0.604 ± 0.210 | 16.8 ± 4.7 |
| | (0.36 – 1.90) | (0.325 – 1.625) | (4.7 – 36.2) |
| | *n* = 217 | *n* = 46 | *n* = 229 |
| mg/kg per day | 7.43 ± 3.57 | 8.02 ± 3.85 | 270 ± 65 |
| | (2.96 – 11.1) | (2.82 – 15.7) | (230 – 419) |
| Literature | | | |
| µmol/8 h | | 1.7 ± 0.2 [4] | 19.3 ± 1.2 [4] |
| mg/kg per day | 10 [5] | | |

Values are the mean ± SD (range). Oxalate data compiled both on enzymatic determinations and the colorimetric method of Hodgkinson [*4*], Kumar et al. (1991); [*5*], Hodgkinson (1977)

were determined by atomic absorption spectrophotometry (AA). Magnesium and calcium were also analyzed by AA, but with the addition of 0.5% lanthanum in the diluent [15]. On alternate unacidified urines, an aliquot was taken for determination of citric acid [16], creatinine [17], and protein [18].

Urines collected on ice were 0.45 µm-filtered and ultra-filtered using an Amicon YM-10 membrane to remove interfering substances of less than 10000 molecular weight [19]. Retentates were resuspended in 20% glycerol in sterile normal saline [20]. Retentates were analyzed for the following enzymes: γ-glutamyl transpeptidase, EC 2.3.2.2 (GGT) using L-glutamyl-*p*-nitroanilide as a substrate and glycylglycine as an acceptor [21]; alkaline phosphatase, EC 3.1.3.1 (AIP) [22], and acid phosphatase EC 3.1.3.2 (AcP) [23] both using *p*-nitrophenyl phosphate as a substrate; leucine aminopeptidase, EC 3.4.1.1 (LAP) using L-leucine-*p*-nitroanilide as a substrate [24]; *N*-acetyl-β-glucosaminidase, EC 3.2.1.30 (NAG) using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide and β-galactosidase, EC 3.2.1.23 (GAL) using 4-nitrophenyl-N-β-D-galactopyranoside, as described by Maruhn [25]. Values are expressed as international unit (U) excretion a 24-h collection period. 1 U equals the amount of enzyme that catalyzes the conversion of 1 µmol substrate per minute under the assay conditions. All analyses were performed in duplicate.

## Results

Urinary values are presented in Tables 1 – 4. Table 1 does not include a literature reference for the turbidimetric analysis of sulfate. Our MEDLINE searches through 1966 failed fo produce a single reference for the male Sprague-Dawley rat.

## Discussion

The information presented here basically constitutes raw data, in that the urinary species are initially expressed in millimoles per liter, rather than as a measure based upon creatinine, protein, or weight. Some tables do include 24 h values normalized by weight. Since measurements of protein and creatinine can vary from laboratory to laboratory and from analysis to analysis, we have included the raw data as well as our values for these species. By presenting data in this form, we feel it allows researchers an easier point of reference for their own data. Since the tables include daily urinary volume and all rat weights fall within a 50 g range, almost any coarse manipulation of the data should be possible.

The animals in our experiments were fed normal rat chow and deionized water, ad libitum, thus eliminating the effects of dietary restrictions and regional or seasonal tap water variations. Again, collections were made on a 24 h basis, eliminating any skew due to the nocturnal nature of the rat. Since rats eat and exercise almost exclusively at night, analysis of urine collections made during daylight hours will vary significantly from 24 h collections. Further, in our experience prolonged confinement of the normal control rat to the metabolic cage causes several alterations in urinary chemistries including an increased excretion of protein and creatinine and brush-border-derived enzymes. To normalize data properly we find that all experiments necessitate a concurrent pair-fed control group rather than only a baseline collection from each experimental group. The data reported above originated from a baseline collection after a 4-day acclimatization period to the cages.

Any variation between our laboratory results and those of another laboratory could be due to any number of circumstances, and any attempt to explain such differences would be purely speculative. Wide dissimilarities are evidenced in both Mitruka [1] and Loeb [2]. This may be due to any of the factors mentioned in the introduction, including age of the rat, strain, dietary intake, meth-

**Table 3.** Urinary creatinine, protein, volume and pH

| Source | Creatinine (units as indicated) | Protein (units as indicated) | Volume (units as indicated) | pH |
|---|---|---|---|---|
| UF | 105.4 ± 27.3 mg/dl<br>(78.6 – 190.0)<br>$n = 174$ | 191 ± 169 mg/dl<br>(13 – 501)<br>$n = 174$ | 15.6 ± 3.2 ml/day<br>(7.6 – 33.0)<br>$n = 263$ | 7.12 ± 0.28<br>(6.35 – 8.20)<br>$n = 263$ |
| | 19.4 ± 5.8 mg/24 h<br>(8.0 – 28.9) | 8.3 ± 5.7 mg/24 h<br>(2.31 – 25.1) | 67.8 ± 16.0 ml/kg per day<br>(22.6 – 172.6) | |
| Literature | 0.45 ± 0.12 mg/dl [1]<br>(24.0 – 40.0) mg/24 h [1] | (30 – 400) mg/dl [2]<br>(5 – 20) mg/24 h [2] | 9.3 – 12.3 ml/day [2]<br>150 – 350 ml/kg per day [1] | 7.7 – 8.1 [2]<br>7.30 ± 8.50 [1] |

Twenty-four urinary values for unacidified samples. Values are the mean ± SD (range)
[*1*], Mitruka and Rawnsley (1977); [*2*], Loeb and Quimby (1989)

**Table 4.** Activities of urinary lysosomal enzymes acid phosphatase (*AcP*), *N*-acetyl-β-glucosaminidase (*NAG*), and β-galactosidase (*GAL*) and membrane enzymes γ-glutamyl transpeptidase (*GGT*), alkaline phosphatase (*AlP*), and leucine aminopeptidase (*LAP*)

| Model | AcP U/24 h | NAG U/24 h | GAL U/24 h | GGT (units as indicated) | AIP (units as indicated) | LAP (units as indicated) |
|---|---|---|---|---|---|---|
| UF | 3.77 ± 0.09<br>(3.03 – 4.77)<br>$n = 40$ | 1.88 ± 0.42<br>(0.58 – 2.28)<br>$n = 89$ | 0.89 ± 0.13<br>(0.76 – 1.06)<br>$n = 43$ | 3.29 ± 1.28 U/24 h<br>(1.91 – 9.05)<br>$n = 53$ | 0.21 ± 0.06 U/24 h<br>(0.07 – 0.32)<br>$n = 98$ | 2.45 ± 0.58 U/24 h<br>(2.00 – 3.75)<br>$n = 53$ |
| Literature | 2.40 ± 2.70 [6] | 1.1 ± 0.4 [6]<br>3.8 ± 0.5 [7] | 2.7 ± 0.9 [6] | 11940 ± 630 U/24 h<br>3.0 ± 0.3 U/24 h [7]<br>0.58 ± 0.09 U/h [9] | 1.10 ± 0.62 U/24 h [6]<br>59.0 ± 5.4 BLU/24 h [8] | 1483 ± 153 U/24 h [6]<br>0.24 ± 0.04 U/h [7] |

Values are the mean ± SD
[*6*], Price (1982); [*7*], Josepovitz et al. (1985); [*8*], Watanabe et al. (1980); [*9*], Gumbleton and Nicholls (1988)

od of collection, processing and storage of samples, or analysis methodology. In addition, expression of enzyme activity is often confusing and many investigators do not hold to the strictest SI definition for international units (IU) of enzym concentration. Frequently these "units" are not defined in the body of the text. Variability may also exist amongst the substrates employed in assays.

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**Editorial comments**

The data presented in this paper provide a comprehensive overview of the urinary chemistry of Sprague Dawley rats. Many of the reported values, however, are substantially different from the (scarce) data published in the literature. Although it was already stated in the paper that many factors may be involved, the usefulness of this report to other investigators will depend on the extent to which experimental conditions contribute to such differences. For instance, it would be of interest to know whether the data presented here may also apply to other strains of rats.

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