# Urinary Excretion of Acetaminophen by the Rat

**Keyphrases** □ Acetaminophen—urinary excretion by the rat, suitability of the rat as an experimental animal □ Metabolism—urinary excretion of acetaminophen by the rat, suitability of the rat as an experimental animal □ Excretion, urinary—acetaminophen by the rat, rat-human metabolic patterns compared

*To the Editor:*

Acetaminophen, after a therapeutic dose, is excreted in the urine of humans primarily as the glucuronic acid conjugate (1, 2). Büch *et al.* (3) reported that acetaminophen glucuronide was also the major metabolite excreted in the urine by rats 4 hr after administration of large doses of acetaminophen (300 and 600 mg/kg). Recently, the rat was used to investigate a potential interaction between relatively low doses of acetaminophen (10 mg/kg) and salicylic acid (4). We are not aware of reports concerning the metabolism and excretion of acetaminophen in rats after lower doses, which are more comparable to those used therapeutically in humans. It was of interest, therefore, to determine whether the rat excreted acetaminophen in a manner similar to that reported for humans.

We determined the amounts of acetaminophen and its glucuronide and sulfate conjugates excreted in the urine of two strains of rats given a 25-mg/kg dose of acetaminophen. An aqueous solution of acetaminophen, 2.5 mg/ml, was administered orally or intraperitoneally to male Wistar rats (290–340 g) and intraperitoneally to male Sprague–Dawley rats (290–370 g). Urine for analysis was collected for 24 hr. Preliminary experiments showed that excretion of the drugs was essentially completed within this period. Acetaminophen and its conjugates in urine were determined using selective enzyme hydrolysis followed by GLC analysis. The conjugates were hydrolyzed enzymatically, and acetaminophen was extracted with ether using a modification of the method of Levy and Yamada (2). This procedure was followed by back-extraction into 0.1 *N* NaOH, with subsequent removal of the organic phase and neutralization of the aqueous phase to pH 7.0. Acetaminophen was extracted from the aqueous solution with ethyl acetate. The organic layer was evaporated to dryness, and the acetaminophen was derivatized with bis(trimethylsilyl)trifluoroacetamide[1] and was analyzed by GLC using a modification of the method of Thomas and Coldwell (5).

The results (Table I) indicate that, after a dose of 25 mg/kg, both strains of rats excreted acetaminophen principally as the sulfate conjugate. A very small percentage of the dose was excreted as the glucuronide conjugate or as unchanged drug. The GI tract is an important site for formation of glucuronic acid conjugates (6). Acetaminophen was administered orally to rats to determine if the low amount of acetaminophen glucuronide excreted in the urine following an intraperitoneal dose of acetaminophen was due to the unavailability of this site of glucuronidation. Acetaminophen was probably not conjugated with glucuronic acid during absorption because oral administration resulted in no change in the urinary excretion of the drug when compared to the results of intraperitoneal administration.

These data from rats differ considerably from urinary excretion data following administration of acetaminophen to humans. Cummings *et al.* (1) reported that 49% of a 12-mg/kg dose was excreted as acetaminophen glucuronide, with smaller amounts excreted as acetaminophen sulfate (25%) and unconjugated drug (4%). Levy and Yamada (2) reported similar results for humans following 1- or 2-g doses (63% as acetaminophen glucuronide, 26% as acetaminophen sulfate, and 3% as unconjugated drug). We observed in three humans that 47.8 ± 3.8% (±*SEM*) of a 10-mg/kg dose of acetaminophen was excreted as the glucuronide conjugate while 32.3 ± 5.9% of the dose was excreted as acetaminophen sulfate.

**Table I**—Urinary Excretion of Acetaminophen in the Rat following a 25-mg/kg Dose

| Strain | Route | Percent of Dose[a] Excreted in 24 hr as | | |
|---|---|---|---|---|
| | | Acetaminophen | Acetaminophen Glucuronide | Acetaminophen Sulfate |
| Sprague–Dawley ($n$ = 4) | Intraperitoneal | 1.9 ± 0.3 | 2.7 ± 0.7 | 68.9 ± 6.2 |
| Wistar ($n$ = 4) | Intraperitoneal | 0.8 ± 0.1 | 3.8 ± 0.7 | 68.6 ± 6.5 |
| Wistar ($n$ = 4) | Oral | 1.2 ± 0.2 | 4.8 ± 0.7 | 77.0 ± 7.4 |

[a] Expressed as the mean ± standard error.

Büch *et al.* (3) reported a saturation of the sulfate-conjugating pathway in the rat following 300- and 600-mg/kg doses of acetaminophen. Levy and Yamada (2) suggested that the pathway for sulfate conjugation in humans was saturated at doses of 12–32 mg/kg. The differences between the urinary metabolite pattern in rats given high doses of acetaminophen and that in rats treated with a 25-mg/kg dose may be due to saturation of the sulfate-conjugating pathways at high dosages, with a resultant increase in acetaminophen glucuronide formation.

We conclude from these studies that the rat is not an appropriate animal model for acetaminophen me-

[1] Regisil, Regis Chemical Co., Chicago, Ill.

tabolism in humans. Acetaminophen appears to be excreted in the urine primarily as acetaminophen sulfate when administered to rats in doses comparable to human doses. In contrast, the glucuronide conjugate constitutes the largest fraction of the dose of acetaminophen excreted in the urine by humans. Therefore, results from studies of the interference by other drugs upon acetaminophen conjugation in the rat cannot be readily extrapolated to similar situations in humans. This possibly explains why Ramachander *et al.* (4) observed an interaction between acetaminophen and salicylate in rats while Levy and Regårdh (7) could not demonstrate this in humans.

(1) A. J. Cummings, M. L. King, and B. K. Martin, *Brit. J. Pharmacol. Chemother.*, **29**, 150(1967).

(2) G. Levy and H. Yamada, *J. Pharm. Sci.*, **60**, 215(1971).

(3) H. Büch, K. Pfleger, and W. Rüdiger, *Z. Klin. Chem. Biochem.*, **5**, 110(1967).

(4) G. Ramachander, F. D. Williams, and J. F. Emele, *J. Pharm. Sci.*, **62**, 1498(1973).

(5) B. H. Thomas and B. B. Coldwell, *J. Pharm. Pharmacol.*, **24**, 243(1972).

(6) T. A. Miettinen and E. Leskinen, in "Metabolic Conjugation and Metabolic Hydrolysis," vol. I, W. H. Fishman, Ed., Academic, New York, N.Y., 1970, pp. 170, 171.

(7) G. Levy and C. Regårdh, *J. Pharm. Sci.*, **60**, 608(1971).

*R. P. Miller*
*L. J. Fischer*[x]
Toxicology Center
Department of Pharmacology
University of Iowa
Iowa City, IA 52242

Received January 31, 1974.
Accepted for publication April 4, 1974.
[x] To whom inquiries should be directed.

## Decomposition of Cannabidiol in Chloroform Solution

**Keyphrases** □ Cannabidiol—stability in chloroform and ethanol, GLC, TLC □ Stability—cannabidiol in chloroform and ethanol, GLC, TLC

*To the Editor:*

Concurrent with our investigation of the stability of the cannabinoids, we observed what appeared to be an unusually large loss of cannabidiol when stored in a chloroform solution. Since chloroform is frequently employed in cannabinoid extraction and analysis (1–10), we felt it imperative to investigate the stability of cannabidiol in this solvent. Previous reports on the stability of cannabinoids in solution were concerned primarily with crude marijuana extracts. Kubena *et al.* (11) reported that cannabidiol was one of the cannabinoids present in a 43-year-old alcoholic solution of marijuana extract, and Turner *et al.* (12) recently indicated that the extractable cannabinoids, including cannabidiol, are stable in chloroform for at least 6 days. However, we wish to report a marked decomposition of cannabidiol in chloroform as compared to its relative stability in ethanol.

**Table I**—GLC Analysis[a] of Cannabidiol in Ethanol and in Chloroform

| Day | Cannabidiol Peak Area[b] | | Cannabidiol–Steroid Ratio[c] | |
|---|---|---|---|---|
| | A(Ethanol) | B(Chloroform) | C(Ethanol) | D(Chloroform) |
| 0 | 100 | 100 | 1.00 | 1.00 |
| 1 | 105 | 91 | 1.02 | 0.88 |
| 2 | 85 | 61 | 0.97 | 0.68 |
| 3 | 80 | 54 | 0.96 | 0.56 |
| 4 | 85 | 34 | 0.89 | 0.31 |
| 5 | 82 | 23 | 0.97 | 0.19 |
| 6 | 86 | 14 | 0.96 | 0.09 |
| 7 | 87 | 9 | 0.97 | 0.04 |
| 8 | 84 | 5 | 0.96 | 0.01 |

[a] Average of two or three determinations where integrated peak area differences were not greater than 8%. [b] Percent relative to the peak area on Day 0. [c] Normalized to 1.00 on Day 0; actual values on Day 0 were 1.10 for C and 1.05 for D.

Four 1-ml solutions, each containing 1.0 mg of cannabidiol[1], were prepared: Solution A, in absolute ethanol; Solution B, in spectrograde chloroform[2]; Solution C, in absolute ethanol containing 1.0 mg 4-androstene-3,17-dione[3] (steroid) as an internal standard (7–9); and Solution D, in spectrograde chloroform containing 1.0 mg of steroid as an internal standard. Except during analysis, the solutions were stored at room temperature in the dark in tightly stoppered 1-ml volumetric tubes.

The GLC analysis was performed using a gas chromatograph[4] equipped with a digital integrator[5] and dual 0.6-cm (0.25-in.) by 1.8-m (6-ft) glass columns packed with 3% OV-17 on 100–120-mesh Gas Chrom Q. The experimental conditions were: injection port temperature, 232°; detector temperature, 240°; column temperature, 218°; and nitrogen flow rate, 50 ml/min[6]. Dual flame-ionization detectors were employed, and the attenuation was adjusted so that a 1.0-$\mu$l sample of each solution produced a cannabidiol peak of approximately 70% full-scale deflection on Day 0.

The data (Table I) show that there was a significant decrease in the cannabidiol content in chloroform, even in the first 24 hr, while the cannabidiol content in ethanol remained essentially unchanged. The GLC data of Solutions A and B represent absolute values of peak areas and are subject to the usual experimental variations[7]. The consistency of the cannabidiol to steroid ratio in Solution C indicates that the cannabidiol content in ethanol is relatively stable and does not decrease as might be concluded from the data for Solution A alone.

The TLC technique employed was that of Korte *et al.* (10). After impregnation with dimethylformam-

[1] Received from Dr. Monique Braude, Department of Health, Education, and Welfare, U.S. Public Health Service, National Institute of Mental Health, Bethesda, Md.
[2] Fisher Scientific, Fair Lawn, N.J.
[3] E. M. Laboratories Inc., Elmsford, N.Y.
[4] Perkin-Elmer model 990.
[5] Infotronics.
[6] Retention times: cannabidiol, 6.1 min; and steroid, 17.1 min.
[7] Absolute peak area stability could not be maintained because of the necessity of daily removal of the columns from the instrument.