levels[1]. A boiling alkaline picrate method claimed for the determination of "true" creatinine has also been shown to give an average 32.4 ± 27.2% (*SD*) overestimation, with the highest overestimation being 102% (7).

We did not advocate that our method be used routinely for all creatinine determinations in patients. Since plasma or serum samples as small as 10 μl are sufficient in our method, we suggested that the method would be "particularly valuable to the monitoring of the renal function in premature and mature infants, children, and adults with collapsed vascular veins. In these patients, the sample required can be obtained easily from the fingertip or the heel in the case of tiny, premature infants."

Place misunderstood our paper regarding the analysis time for each sample. We stated in the Abstract that: "Each assay required only about 5 min for completion." This statement was mistaken by Place to mean 5 min per chromatographic time. In fact, we meant that the time from the receipt of a plasma or serum sample to the reporting of the creatinine level of the sample is ~5 min. Less time is required for multiple-sample analyses. This time compares favorably with the membrane method, which requires ~90 min for emergency cases. The proposed HPLC method can certainly be modified and automated. The potential clinical application of the HPLC method for creatinine assay was examined in two recent papers (5, 6).

Regarding Place's (2) comment on the work of Moss *et al.* (8), it should be pointed out that the lowest creatinine concentration shown in their standard curve is 2 mg %, although the use of a 1 mg % solution was mentioned in the text. Certainly, it is not most desirable to perform the reproducibility study using the creatinine level below the range used for the standard curve.

Although the data on reproducibility of measurements of concentrations <1 mg % were not presented explicitly in our papers (1, 7), they were clearly implied. Excellent reproducible results were always obtained for low creatinine levels in our many standard curve and plasma level studies (1, 7). Using a modified mobile phase and a 254-nm fixed wavelength detector, the coefficient of variation for 0.5 mg % serum sample is only about 2% (9). Our comment (1) on the interferences in samples from ketotic patients should have been directed to the colorimetric method of Heinegård and Tiderström (10) and not to the ion-exchange membrane method.

It is important to point out that in using our HPLC method (1) for creatinine, the filter device[2] to separate plasma or serum from blood cells should not be used; chemicals leached out from the device will interfere with the assay. The possibility of such interference in other HPLC methods should be studied prior to their use.

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[1] Unpublished data.
[2] Filter sampler blood serum filter, Glassrock Products, Fairburn, GA 30212.

Effect of Sodium Sulfate on Acetaminophen Elimination by Rats

Keyphrases □ Sodium sulfate—effect on acetaminophen elimination, protection against acetaminophen toxicity, rats □ Acetaminophen—toxicity, prevention by sodium sulfate, effect on elimination □ Analgesics—acetaminophen, toxicity, prevention by sodium sulfate

To the Editor:

Accidental or intentional ingestion of large acetaminophen overdoses can cause hepatotoxicity and damage to other vital organs, sometimes with lethal outcome (1, 2). This toxic effect is produced by a reactive metabolite, which is formed in parallel with acetaminophen glucuronide and acetaminophen sulfate, the two major drug metabolites (3). Pharmacokinetic analysis of acetaminophen disposition in acutely intoxicated patients and certain other evidence from studies in humans indicate that the reactive acetaminophen metabolite is formed by apparent first-order kinetics while the two major conjugated metabolites are formed by capacity-limited kinetics (4, 5). Results of acetaminophen conjugation interaction studies in humans suggest that the availability of sulfate is rate limiting in the formation of acetaminophen sulfate (6, 7). If the formation of acetaminophen sulfate can be accelerated by administration of sodium sulfate or another suitable sulfate source, then the amount of reactive metabolite formed and, therefore, the acetaminophen toxicity should be decreased.

Preliminary studies in mice showed that intraperitoneal administration of sodium sulfate significantly increased the median lethal dose (*i.e.*, decreased the toxicity) of acetaminophen (8), but the effect of sodium sulfate on acetaminophen disposition was not determined. There is some indication that acetaminophen sulfate formation by rats is capacity limited and that treatment with sodium sulfate increases the urinary excretion of the conjugate (9, 10). However, the reported data do not establish definitively the effect of sodium sulfate on acetaminophen elimination kinetics since acetaminophen concentrations in plasma were not determined and urine collections were terminated before the excretion of drug and metabolites was completed.

In view of the potential clinical utility of sodium sulfate

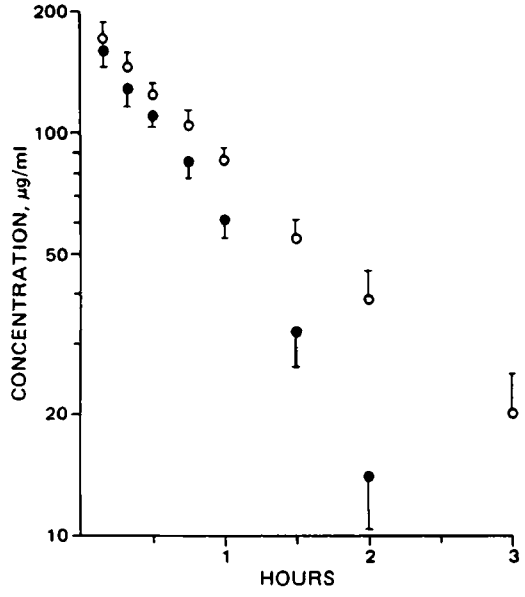

Figure 1—*Effect of sodium sulfate on elimination of acetaminophen, 150 mg/kg iv, by male Sprague–Dawley rats. Sodium sulfate was administered intraperitoneally (142 mg/kg 15 min before acetaminophen) and by intravenous infusion (about 21 mg/kg/hr for 2 hr, starting at the time of acetaminophen injection). Shown are the acetaminophen concentrations in plasma (mean + or − SD, n = 7) as a function of time in control (○) and sulfate-treated (●) animals. Concentrations differed significantly (p < 0.02) from 20 min on.*

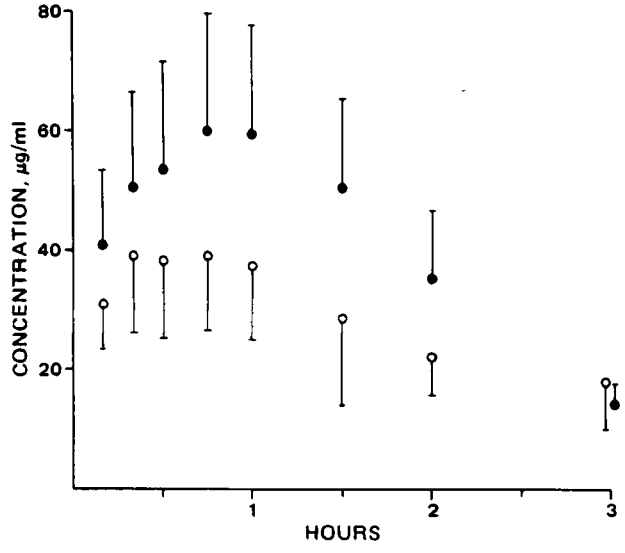

Figure 2—*Acetaminophen sulfate concentrations (expressed in terms of acetaminophen) as a function of time in plasma of control (○) and sodium sulfate-treated (●) rats (mean + or − SD, n = 7). The areas under the two curves from zero time to 3 hr differed significantly (p < 0.05).*

for preventing or reducing acetaminophen toxicity (5), a comprehensive study of the effect of sodium sulfate on acetaminophen elimination kinetics has been instituted. Reported here are results of a preliminary study which showed that sodium sulfate does substantially accelerate acetaminophen elimination in rats.

Fourteen adult male Sprague–Dawley rats, 280–320 g, had a cannula implanted in the right jugular vein (11, 12) 1 day before the study. Food and water were withdrawn in the morning at the beginning of the experiment. Acetaminophen, 150 mg/kg (25 mg dissolved in 1 ml of 40% propylene glycol in water at 40°), was injected intravenously. Seven animals received a bolus dose of sodium sulfate, 142 mg in 3 ml of water/kg ip 15 min before acetaminophen and an intravenous sodium sulfate infusion, 142 mg in 10 ml of water, over 2 hr beginning at the time of acetaminophen injection. Seven control rats received an intraperitoneal injection and an intravenous infusion of sodium chloride solution containing the same sodium concentrations as the sodium sulfate solutions.

Blood samples (0.45 ml) were obtained periodically over 4 hr. Plasma was separated and assayed for acetaminophen and acetaminophen sulfate by high-performance liquid chromatography. The assay was a modification of a published procedure (13) with the following changes: μBondapak C-18 column[1], 4-fluorophenol as the internal standard, 2-ml/min flow rate, isopropanol in the mobile phase reduced to 1.0 part, and 50% trichloroacetic acid (1 part/2 parts of plasma) for plasma protein precipitation.

Sodium sulfate caused plasma acetaminophen concentrations to decline much more rapidly in the experimental animals than in control rats (Fig. 1). Other data, to be reported later, show that acetaminophen elimination is dose dependent even though the plasma drug concentration decline is apparently exponential. The time required for acetaminophen concentrations to decrease by 50% was 55 ± 7 min in control rats and 32 ± 5 min in sodium sulfate-treated rats (mean ± *SD*, $p < 0.001$) under the experimental conditions. Conversely, plasma acetaminophen sulfate concentrations were significantly higher in sodium sulfate-treated than in control animals (Fig. 2).

This study demonstrated that sodium sulfate increases acetaminophen sulfate formation and thereby accelerates acetaminophen elimination in rats. Inferentially, such treatment should reduce the formation of other metabolites by competing pathways, particularly those, like the reactive acetaminophen metabolite, that are formed by apparently linear kinetics. These conclusions are consistent with the observation that sodium sulfate reduces acetaminophen toxicity in mice (8). If clinical studies, now in progress, yield similar results, then incorporating sodium sulfate or another sulfate source in pharmaceutical products containing acetaminophen (14) will deserve serious consideration.

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BOOKS

REVIEWS

Essentials of Toxicology, 3rd Ed. By TED A. LOOMIS. Lea & Febiger, 600 Washington Square, Philadelphia, PA 19106. 1978. 245 pp. 15 × 24 cm. Price $12.50.

The newest edition of this basic toxicology text differs little from the previous edition in both form and content. The type is slightly larger and thus easier to read. Many chapters have few, if any, alterations. Some of the additional or revised material to be found includes: updated statistics on poisoning and mortality from chemical exposure; an amplified section on the influence of the microsomal P-450 enzyme system on chemical toxicity; a table of genetically based alterations which account for individual variations in response to drugs and chemicals; a section differentiating the concepts of biological half-life of a compound *versus* "half-life for toxicity," using as an example fatty deposition in liver after exposure to ethanol; and a brief description of behavioral toxicity studies in animals.

A table on page 60 lists formaldehyde as the toxic metabolite of methanol, although recently published data indicated that formate, and not formaldehyde, is responsible for ocular toxicity in monkeys and presumably in humans.

Each chapter is adequately referenced to provide the interested student with sources of more detailed information. This book cannot be compared to other texts such as Casarett and Doull's "Toxicology, The Basic Science of Poisons" (Macmillan) or portions of Goldstein, Aronow, and Kalman's "Principles of Drug Action" (Wiley), both of which deal more extensively with toxicologic subject matter. However, "Essentials of Toxicology" provides a well-organized introductory approach to toxicology and can serve a useful purpose in an orientation course to this highly diversified science.

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Blood Drugs and Other Analytical Challenges (in Methodological Surveys in Biochemistry), Vol. 7. Edited by E. REID. Ellis Horwood Ltd., Market Cross House, 1 Cooper Street, Chichester, Sussex, England (U.S. distributor: Halstead Press, a division of John Wiley & Sons, 605 Third Avenue, New York, NY 10016). 1978. 355 pp. 14.8 × 22.6 cm. Price $47.50.

This volume (Volume 7) compliments its predecessor (Volume 5) and is based on the papers presented at Bioanalytical Forum held at the University of Surrey in 1977. The book provides practical information of value to the bioanalytical researcher and attempts to present methodological rationale rather than mere recipes. The first four chapters are subdivided into sections, and the fifth chapter represents the notes, comments, and discussion in the form of questions and answers on the preceding four chapters.

The first chapter, "The Framework," sets out the general philosophy for development of analytical methods, discusses quality control and sources of errors in assays, and presents an overview on analytical method evaluation. The second chapter describes aspects of gas chromatography (GLC) with problems associated with capillary and packed columns and detectors (AFID and ECD). Their applications in drug analysis as well as derivatization procedures are discussed also.

In the third chapter, mass spectrometric methods for drug and endogenous compound analyses in biological fluids are presented, with suitable examples, together with considerations concerning accuracy and precision. Discussions of more recent approaches in this field such as negative-ion mass spectrometry and HPLC—mass spectrometry are particularly valuable.

The fourth chapter discusses the applications of HPLC to drug analysis. Interesting and useful discussions with relevant examples are provided on ion-pair HPLC of acid and basic drugs, metabolites, and endogenous compounds. Various aspects of HPLC such as electrochemical detection, sample handling, chemical derivatization including fluorescence labeling, and prechromatographic methods in biomedical trace analysis are presented with useful comments and suitable examples.

The analytical case histories on the assays of bendrofluzide, biperidin, and labetalol and its metabolites are interesting and informative. The notes and comments in the fifth chapter along with analytical case histories of drugs including metoclopramide, procetofenic acid, tienlilic acid, practolol, amitriptyline, nortriptyline and benzodiazepines make particularly enjoyable reading for the analyst.

In summary, the reviewer found this book to be a well-balanced blend of the theoretical and practical aspects of present techniques and their potential applications of trace drug analysis in biological fluids.

The book is effective in its scope, variety of experiments, and presentation style. It is well planned, emphasizes the rationale of developing successful analytical methods for drug analysis by chromatographic techniques, and should be useful to analytical chemists working with biological fluids.

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Lange's Handbook of Chemistry, Twelfth Edition. Edited by JOHN A. DEAN. McGraw-Hill, New York, NY 10020. 1978. 1470 pp. 15 × 23 cm. Price $28.50.

This new edition of *Lange's Handbook of Chemistry* is a valuable updating of a classic one-volume reference.

A major improvement on the previous edition is the revised section on thermodynamics. The new section reflects currently recommended values for heats of formation and Gibbs energies of formation, entropies, heat capacities at five different temperatures, and heats of melting, vaporization, and sublimation for 2400 inorganic and 1500 organic compounds.