carcinogenic or tumor-promoting activity of the cyclobutylsulfamate and cyclopentylsulfamate, and possibly the cyclopropylsulfamate, might be worthwhile.

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Determination of Trimethoprim and Its *N*-Oxide Metabolites in Urine of Man, Dog, and Rat by Differential Pulse Polarography

Keyphrases ☐ Trimethoprim and *N*-oxide metabolites—analysis in urine of man, dog, and rat, differential pulse polarography ☐ Polarography, differential pulse—analysis, trimethoprim in urine of man, dog, and rat

Sir:

An analytical method was recently reported for the determination of trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] in blood and urine of man and dog by differential pulse polarography (1). It was qualitatively noted that a portion of the dose was excreted as the unconjugated N_1-oxide, N_3-oxide, and hydroxy-methyl metabolites of trimethoprim, all of which are resolved by TLC. However, due to low recoveries, the measurement of these metabolites was not previously reported. The purposes of this work were to quantitate the *N*-oxide metabolites and to determine if a species difference existed regarding the formation of the *N*-oxides. Since recovery of the hydroxy-methyl metabolite remained low with this modified assay; its measurement is not reported.

A modification of the above-mentioned assay is reported, which includes a "salting-out" procedure to facilitate the extraction of the *N*-oxides into chloroform. In the modified procedure, 1 ml. urine and 4 ml. distilled water are added to a 50-ml. centrifuge tube to which 6 g. anhydrous potassium carbonate is then added. The method from this point onward is identical to the previously reported one (1), with the exception that polarography is performed in 1 *M* pH 3.0 phosphate buffer rather than in 0.1 *N* H_2SO_4. Five and ten micrograms of trimethoprim and the *N*-oxides added to urine as internal standards should be processed with the unknowns. The choice of pH 3.0 phosphate buffer for the urinary assay is dictated by the need for maximal separation in potential of the two *N*-oxide peaks for qualitative identification. This supporting electrolyte was completely free of background interferences in the region of the polarographic reduction of the *N*-oxides.

Polarography is a useful means of identifying and quantitating the *N*-oxides (Fig. 1). The *N*-oxides of trimethoprim possess two distinct polarographic peaks at −1.095 and −0.950 v. *versus* a saturated calomel electrode (SCE) in 1 *M* pH 3 phosphate buffer due to the reduction of the N_1- and N_3-oxide functional groups, respectively. These peaks are easily distinguished from the reduction peak of the azomethine bond in the pyrimidine ring of all three compounds, which occurs at −1.190 v. *versus* SCE in the pH 3.0 phosphate buffer. Although each *N*-oxide possesses a distinct functional group, the *N*-oxide compounds cannot be determined without prior separation because large quantities of trimethoprim in the urine result in a large analytical peak that masks the *N*-oxide peaks. Thus, TLC separation, in which the compounds are identified by comparison with the R_f values of the authentic compounds

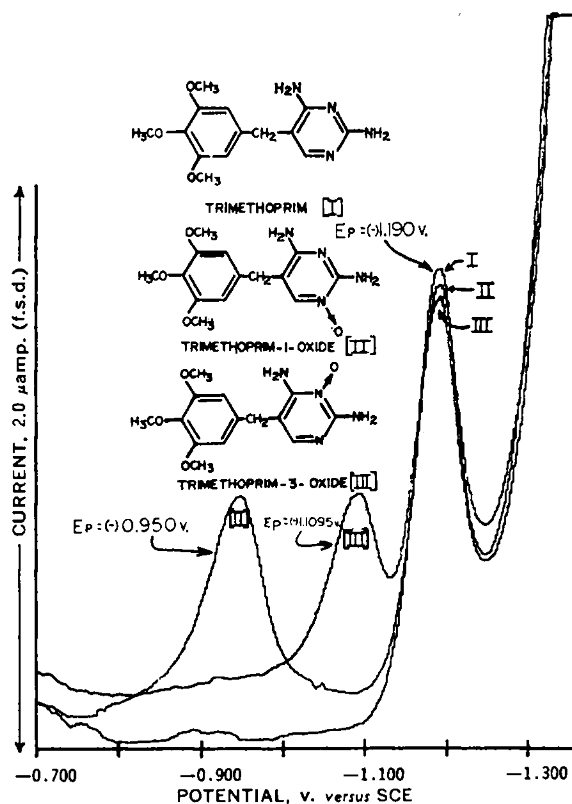

Figure 1—*Polarograms of trimethoprim and its* N*-oxide metabolites in 1* M *phosphate buffer (pH 3).*

Table I—Urine Levels of Trimethoprim and Trimethoprim N-Oxides in Man following Oral Administration[a]

	Trimethoprim 0–24 hr.	Trimethoprim 24–48 hr.	Trimethoprim 48–72 hr.	Trimethoprim N_1-Oxide 0–24 hr.	Trimethoprim N_1-Oxide 24–48 hr.	Trimethoprim N_1-Oxide 48–72 hr.	Trimethoprim N_3-Oxide 0–24 hr.	Trimethoprim N_3-Oxide 24–48 hr.	Trimethoprim N_3-Oxide 48–72 hr.	Total
Subject 1:										
Total milligrams equivalent to trimethoprim	118.4	122.0	16.9	3.6	6.0	n.m.[b]	9.1	n.m.	n.m.	276.0
Percent dose	29.6	30.5	4.2	0.9	1.5	—	2.3	—	—	69.0
Subject 2:										
Total milligrams equivalent to trimethoprim	178.9	34.9	s.n.t.[c]	6.6	n.m.	s.n.t.	4.1	n.m.	s.n.t.	224.5
Percent dose	44.7	8.7	—	1.6	—	—	1.0	—	—	56.0

[a] Total dose was 400 mg. trimethoprim (5.7 mg./kg.). Five tablets were given orally (each tablet contained 400 mg. sulfamethoxazole and 80 mg. trimethoprim). [b] n.m. = nonmeasurable. [c] s.n.t. = sample not taken.

Table II—Urine Levels of Trimethoprim and Trimethoprim N-Oxides in the Dog following Oral Administration[a]

	Trimethoprim 0–24 hr.	Trimethoprim 24–48 hr.	Trimethoprim N_1-Oxide 0–24 hr.	Trimethoprim N_1-Oxide 24–48 hr.	Trimethoprim N_3-Oxide 0–24 hr.	Trimethoprim N_3-Oxide 24–48 hr.	Total
Dog 1:							
Total milligrams equivalent to trimethoprim	32.7	5.6	0.8	n.m.[b]	6.5	1.1	46.7
Percent dose	16.3	2.7	0.4	—	3.2	0.6	23.2
Dog 2:							
Total milligrams equivalent to trimethoprim	48.8	9.5	0.6	n.m.	10.0	1.8	70.7
Percent dose	24.4	4.7	0.3	—	5.0	0.9	35.3

[a] Total dose was 200 mg. trimethoprim (16 mg./kg.). Two and one-half tablets were given orally (each tablet contained 400 mg. sulfamethoxazole and 80 mg. trimethoprim). [b] n.m. = nonmeasurable.

run alongside, is required before quantitative determination by polarography.

The modified method gives recoveries of the N_1- and N_3-oxide of 36.3 ± 8.4 and 59.1 ± 7.5 (*SD*), respectively, in the concentration range of 5.0–30.0 mcg. However, due to the salting out, large amounts of biological interferences are coextracted with the compound of interest. Since the biological interferences tend to make recognition of the zones on the TLC plate difficult, zones are chosen by R_f, *i.e.*, 0.16, 0.25, and 0.45 for N_1-oxide, N_3-oxide, and trimethoprim, respectively. To ensure that no overlap of the N-oxide zones occurs, the plates may be redeveloped using the same solvent system. Thus, this modified method is recommended only when metabolite information is required. For the determination of trimethoprim, the previous method (1) is preferred.

Trimethoprim was administered to man, dog, and rat, and the amounts of intact drug and N-oxides were measured as already outlined. The urines from two human subjects receiving tablets containing a total of 2000 mg. sulfamethoxazole and 400 mg. trimethoprim (5.7 mg. trimethoprim/kg. body weight) were analyzed for trimethoprim and its N-oxide metabolites (Table I).

Table III—Urine Levels of Trimethoprim and Trimethoprim N-Oxides in the Rat following Intraperitoneal Administration[a]

	Trimethoprim 0–24 hr.	Trimethoprim 24–48 hr.	Trimethoprim N_1-Oxide 0–24 hr.	Trimethoprim N_1-Oxide 24–48 hr.	Trimethoprim N_3-Oxide 0–24 hr.	Trimethoprim N_3-Oxide 24–48 hr.	Total
Rat 1:							
Total milligrams equivalent to trimethoprim	2.4	0.07	0.7	n.m.[b]	n.m.	n.m.	3.2
Percent dose	8.5	0.3	2.7	—	—	—	11.4
Rat 2:							
Total milligrams equivalent to trimethoprim	1.4	0.08	0.3	n.m.	n.m.	n.m.	1.8
Percent dose	5.0	0.3	1.0	—	—	—	6.3
Rat 3:							
Total milligrams equivalent to trimethoprim	0.55	0.04	0.10	n.m.	n.m.	n.m.	0.69
Percent dose	5.5	0.4	1.0	—	—	—	6.9
Rat 4:							
Total milligrams equivalent to trimethoprim	1.09	0.08	0.06	n.m.	n.m.	n.m.	1.23
Percent dose	10.9	0.7	0.6	—	—	—	12.3

[a] Total dose for Rats 1 and 2 was 28 mg. trimethoprim (140 mg./kg.). For Rats 3 and 4, it was 10 mg. trimethoprim (50 mg./kg.). The dosage form was 2 ml. of a suspension in 5% gum arabic. [b] n.m. = nonmeasurable.

In the two subjects, more than 50% of the administered dose was excreted mainly as intact drug in the 0–48-hr. period. Both the N_1- and N_3-oxides were found in the urine of man in approximately equal quantities, totaling 4.7 and 2.6% of the dose administered in Subjects 1 and 2, respectively. The urines from two dogs receiving tablets containing a total of 1000 mg. sulfamethoxazole and 200 mg. trimethoprim (16 mg. trimethoprim/kg. body weight) were analyzed for trimethoprim and its *N*-oxide metabolites (Table II). In the two dogs, 19.0 and 29.1% of the dose were excreted as intact drug. A significant amount of the N_3-oxide was excreted by both dogs in the 0–48-hr. period, while only a trace amount of the N_1-oxide was excreted. The urines of four rats receiving 28 mg. (140 mg./kg. body weight) and 10 mg. (50 mg./kg. body weight) trimethoprim i.p. in 5% gum arabic were analyzed for trimethoprim and its *N*-oxide metabolites. Between 5 and 12% of the dose was excreted as intact drug at both dose levels. Only the N_1-oxide metabolite was found in the 0–48-hr. rat urine, totaling between 0.6 and 2.7% of the administered dose (Table III).

Schwartz *et al.* (2) studied the metabolism of trimethoprim in man, dog, and rat and found that each species excreted the N_1-oxide as a urinary metabolite; this finding was confirmed in the present study. Meshi and Sato (3) detailed the metabolism of trimethoprim in the rat and reported levels of an *N*-oxide, but they were unable to determine the point of attachment of the oxygen to either nitrogen of the pyrimidine ring. Additional information on the *N*-oxidation of trimethoprim is presented in this study. Although limited numbers of subjects and animals of each species were studied, the results suggest that the human converts trimethoprim to the N_1-oxide and N_3-oxide with equal facility, whereas N_3-oxide formation is favored by the dog and N_1-oxide formation is favored by the rat.

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BOOKS

REVIEWS

Basic and Clinical Pharmacology of Digitalis. (Proceedings of a Symposium sponsored by the Ohio State University College of Medicine, the American Heart Association, and the Central Ohio Heart Chapter). Edited by B. H. Marks and A. M. Weissler. Charles C Thomas, Springfield, IL 62703, 1972. 328 pp. 14.5 × 23 cm. Price $21.00.

The editors successfully present the work of distinguished scientists and clinicians who bring together the most recent advances in basic and clinical aspects of digitalis. In most instances, each of the 16 chapters presents a clear, readable, and succinct picture of the most important facets of digitalis glycosides. The materials throughout the book are well documented with adequate and current references (through 1971).

The application of basic pharmacological concepts to the clinical use of digitalis is the major theme of this book. The last five chapters of Part Two are particularly good and very useful for clinicians. In some instances, different views have been expressed by the authors on the same topic, but this is understandable, since all of these topics can be approached from a different point of view. Inclusion of chemical structures of various digitalis glycosides would have been helpful in following the discussions on the absorption and metabolic transformations of digitalis presented in chapters 2 and 12. However, this is a minor inconvenience since structural information is readily accessible from several reference books.

These authoritative presentations on basic and clinical aspects of digitalis glycosides deserve the careful scrutiny of pharmaceutical and medicinal chemists, practicing cardiologists, and internists, as well as academic and research specialists in cardiovascular physiology and pharmacology and all others who may be obliquely interested with digitalis.

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Amino-Acids, Peptides, and Proteins, Volume 4. Senior Reporter, G. T. Young. The Chemical Society, Burlington House, London, W1V OBN, England, 1973. 498 pp. 13 × 21.5 cm. Price £9.00.

This volume is the fourth in the series of the literature review in the field of amino acids, peptides, and proteins. The selection of the reviewers by the Chemical Society is highly commendable. Under the able leadership of Dr. Young, these specialists have done a wonderful job and have provided an indispensible service by critically reviewing the wealth of the literature during the year 1971. The present volume is divided into five chapters. The first four chapters are devoted to the detailed survey of the literature on Amino-Acids, Structural Investigations of Peptides and Proteins, and Peptide Synthesis and Peptides with structural features not typical of proteins. The fifth chapter, a short one, concerns the revision of the I.U.P.A.C.-I.U.B. recommendations for the nomenclature of amino acids, peptides, and proteins. The second chapter is, by far, the most comprehensive and provides detailed survey of the litera-