

Formation of an Insoluble Condensation Product from Sulfamethizole and Formaldehyde

Sir:

Zinsser, *et al.* (1), recently reported on the composition of the sediment found in human urine after the ingestion of a combination of sulfamethizole, methenamine, and mandelic acid. Using the Bratton and Marshall assay procedure (2), more than 50% of the sediment was shown to be sulfonamide. In agreement with previous investigators (3), the sediment was also stated to contain ammonium salts and urates.

In vitro, we have investigated the compatibility of sulfamethizole, methenamine, and mandelic acid in the pH range of 4.5 to 6.0. It was found that precipitation quickly followed the addition of methenamine to solutions of the sulfonamide. Since methenamine is hydrolyzed to formaldehyde in acidic solution, the aldehyde was tested in the same manner. Formaldehyde precipitated sulfamethizole from solution.

Table I summarizes an experiment involving the addition of formaldehyde, methenamine, and mandelic acid to saturated aqueous solutions of sulfamethizole at pH 5.0 and pH 6.0. The buffer systems employed were 0.1 *M* acetate (pH 5.0) and 0.1 *M* phosphate (pH 6.0). A saturated solution of the sulfonamide was prepared in each buffer at room temperature and the actual concentrations of sulfamethizole were determined by assay (2). [Using a Coleman Junior spectrophotometer and selected round cells as described previously (4), the optical density value corresponding to 1 mcg. of sulfamethizole per milliliter was 0.098.] Ten-milliliter aliquots of each sulfonamide solution were

treated with stoichiometric quantities of mandelic acid and methenamine and with 6 molar equivalents of formaldehyde. Each mixture was filtered at the end of 1 hour and the filtrates were analyzed for sulfamethizole. At both pH levels, sulfamethizole was precipitated quantitatively by formaldehyde in 1 hour. Methenamine caused the precipitation of 22–33% of the sulfamethizole in 1 hour. In a similar experiment conducted at pH 6.0, methenamine was found to precipitate 65–80% of the sulfamethizole in 20 hours.

Druey (5) and Basu (6) independently discovered that sulfathiazole formed the same insoluble condensation product with either formaldehyde or methenamine. From elemental analyses, both workers suggested possible chemical structures for the product. Later, however, new and polymeric structures were advanced for formosulfathiazole (7, 8). An investigation of the nature of the insoluble sulfamethizole-formaldehyde product is in progress at this laboratory. Another area of interest is the determination of the site of formation of the condensation product (kidney, ureter, bladder).

- (1) Zinsser, H. H., Mayer, G. G., Katz, E., and Wang, S., *Am. Chem. Soc. Meeting, Atlantic City, N. J.*, Sept. 9–14, 1962.
- (2) Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, **128**, 537 (1939).
- (3) Garvey, F. K., and Murray, H. L., *N. Carolina Med. J.*, **22**, 203 (1961).
- (4) DiCarlo, F. J., Malament, S. G., Haynes, L. J., and Phillips, G. E., *Toxicol. Appl. Pharmacol.* **4**, 475 (1962).
- (5) Druey, J., *Helv. Chim. Acta*, **31**, 179 (1948).
- (6) Basu, U. P., *J. Indian Chem. Soc.*, **26**, 125 (1949).
- (7) Druey, J., and Becker, A., *Helv. Chim. Acta*, **31**, 2184 (1948).
- (8) Bhatnagar, S. S., and Fernandes, F., *Indian J. Med. Res.*, **38**, 279 (1950).

FREDERICK J. DICARLO
SYLVIA G. MALAMENT
GEORGE E. PHILLIPS

Biochemistry Department
Warner-Lambert Research Institute
Morris Plains, N. J.

Received October 30, 1962.
Accepted for publication November 14, 1962.

TABLE I.—PRECIPITATION OF SULFAMETHIZOLE WITH FORMALDEHYDE AND METHENAMINE

Sulfamethizole, mg./10 ml.	pH	Mandelic Acid, mg.	Methenamine, mg.	Formaldehyde, mg.	Sulfonamide Precipitated, %
6.08	5.0	3.41	0
6.08	5.0	...	3.14	...	27
6.08	5.0	4.08	100
6.08	5.0	3.41	3.14	...	25
16.32	6.0	9.17	0
16.32	6.0	...	8.44	...	22
16.32	6.0	11.0	99
16.32	6.0	9.17	8.44	...	33