sis. Rats fed sulfathiazole show the same syndrome but not as consistently. The calcium of the kidney and muscle of rats fed sulfasuxidine or sulfathiazole is not increased. The altered calcium metabolism of the liver can be reversed or prevented by feeding solubilized liver for 10 to 40 days. Rats receiving sulfadiazine have an increased calcium content of the kidneys but not of liver or muscle.

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The Urinary Excretion of Sulfadiazine Determined with Thiobarbituric Acid, a New Specific Reagent.*

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By means of the thiobarbituric acid reagent described in another connection,[1] 2-aminopyrimidine and its derivatives sulfadiazine and acetylsulfadiazine can be determined quantitatively, the latter being recovered from urine to within 1%. The specificity is such that no reaction was obtained with 15 other pyrimidines, with sulfamerazine, or with sulfanilamide, sulfapyridine, sulfacetamide, sulfathiazole, sulfasuxidine, sulfapyrazine, and sulfaguanidine, with the common constituents of blood and urine, or with 50 control urines.

Procedure. Unknown and standard solutions are prepared to contain 0.5 to 2 mg% of sulfadiazine in 0.2 N HCl. 1 ml of unknown, of standard, and of 0.2 N HCl (the reagent blank) plus 4 ml of thiobarbituric acid reagent[1] are heated on the boiling water bath in chromic acid-cleaned tubes for 45 minutes, evaporation being minimized. The solutions are then made up to 10 ml with water, and within several hours the unknown and standard are read through filter 520 against the reagent blank in the Evelyn photoelectric colorimeter. The Beer-Lambert law is obeyed. The molar extinction coefficients of sulfadiazine and acetylsulfadiazine are equal. Preliminary hydrolysis of the urine (40 minutes, 0.2 N HCl) does not affect the analysis.

Urines from 30 subjects taking sulfadiazine were examined. In 25, the thiobarbituric acid analysis (TBA) of *fresh* urine agreed with the Bratton and Marshall[2] free sulfadiazine (BM-F). But after aging the urine at 5° or 20°C for one week, TBA agreed with the Bratton and Marshall free plus acetylated, or total (BM-T). The same change was obtained in 30 minutes by treating the fresh urine with ascorbic acid or sodium hydrosulfite (final concentration 0.6%). Example: analysis of fresh urine, TBA 61 mg%, BM-F 60.5 mg%, BM-T 91; after treatment with ascorbic acid, TBA 91.5 mg%.

In 5 subjects, however, aging had no effect, TBA agreeing with BM-T from the start. Example: analysis of fresh urine, TBA 113.5 mg%, BM-F 68 mg%, BM-T 115 mg%.

The analysis of a mixture of fresh urines from *both* types of subject equalled the sum of their individual analyses. This indicates that the difference between the types is not due to the presence of an interfering substance in one of them.

Following the ingestion of acetylsulfadiazine, both types of subject reacted in the same manner. De-acetylation was negligible, aging was without effect, and TBA equalled BM-T in fresh urine. Example: analysis of fresh

* Thanks are due the Rockefeller Foundation for a grant in aid of this work, and the American Cyanamid Co. and Sharp and Dohme, Inc., for generous gifts of chemicals.

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2 Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

urine, TBA 81.5 mg%, BM-F 4 mg%, BM-T 81.5 mg%.

That the sulfonamides experience various metabolic changes including oxidations is well known,[3,4,5,6] though the precise nature of these has not been determined in all cases. The present work indicates that the pyrimidine ring in sulfadiazine experiences some change which blocks the TBA test and which can be reversed by reduction. However, when acetylsulfadiazine passes through the body, this change does not occur.

Summary. Thiobarbituric acid is a specific reagent for the determination of sulfadiazine (free or acetylated) among the sulfonamides because it reacts with the aminopyrimidine moiety. In most urines, however, the acetylated fraction which appears following the ingestion of sulfadiazine does not react unless the urine first be aged or be treated with a reducing agent. This suggests that the pyrimidine ring has been oxidized. Acetylsulfadiazine taken by mouth, when subsequently excreted in the urine, does not experience this change.

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A Modification of the Technic for Determination of the Antistreptolysin Titer.*

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The determination of the antistreptolysin titer of human serum has proved to be a valuable tool for use in the study of hemolytic streptococcal infection and related disorders such as rheumatic fever. Its usefulness has been limited by certain technical difficulties inherent in the method most widely used, which was described by Coburn and Pauli,[1] as modified from Todd[2] and Hodge and Swift.[3]

Streptolysin or streptococcus hemolysin is unstable and is readily oxidized to an antigenically potent substance which is unable to hemolyze red blood cells. Previous technics have maintained a lysin for testing purposes in the active state by reduction with sodium hydrosulphite, followed by removal of oxygen in a vacuum, and storage under an oil seal in the cold room. This procedure is cumbersome and deterioration of the lysin occurs rapidly under any but the most favorable circumstances. Furthermore, it has been difficult to regularly produce a lysin of adequate potency because of variations in available culture medias and other factors.

Streptolysin, in the oxidized state, is stable and its hemolytic potency remains unchanged, even though it has been stored for many months in the refrigerator or for relatively long periods at room temperature before reduction. It may also be readily concentrated and partially purified.[4,5,6].

* This investigation was aided through the Commission on Hemolytic Streptococcal Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases, Preventive Medicine Service, Office of the Surgeon General, United States Army.

The work described in this paper was done under contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development, and Stanford University.

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[2] Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267.

[3] Hodge, B. E., and Swift, H. F., *J. Exp. Med.*, 1933, **58**, 277.

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