every second day showed a mean fall in erythrocytes from 9,200,000 to 5,821,000 during 16 days. In some of the fatalities, the erythrocyte count fell to 3,000,000 and less. No evidence of urolithiasis was observed in 30 rats (weighing approximately 200 g) which received 100 mg orally twice daily for 12 days, although 11 animals died within this time.

Sulfadiazine, on the contrary, produced renal failure in a number of infected mice. Since it was impossible to determine whether this was secondary to the damage caused by staphylococci, a series of normal mice and a group of normal rats were treated orally with similar and smaller doses of sulfadiazine. Gross inspection and microscopic examination (frozen sections of fresh tissue) of kidneys showed crystalline deposits, predominantly in the urinary papilla and often sufficiently massive to be capable of completely obstructing the urinary outflow. A more detailed study of this toxic manifestation will be reported elsewhere.[22]

Conclusions. 2-(Sulfanilamido)-5-ethyl-4-thiazolone (sulfaethylthiazolone) has less antistreptococcic activity and approximately the same antipneumococcic activity as sulfapyridine. Its antistaphylococcic activity is of the same order as that of sulfathiazole and sulfadiazine.

Sulfadiazine produces urolithiasis medicamentosa capable of causing death by acute suppression of urine in mice and rats, whereas sulfaethylthiazolone is free of this defect, but may cause fatal anemia.

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Erythrocyte Phosphatase Activity in Hemolysed Sera and Estimation of Serum "Acid" Phosphatases.

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Erythrocytes contain an apparently specific phosphatase[1,2,3] classified by Folley and Kay[4] as phosphomonoesterase A_4. This enzyme

22 Gross, P., Cooper, F. B., and Lewis, M., in press.

1 Martland, M., Hansmann, F. S., and Robison, R., *Biochem. J.*, 1924, **18**, 1152.

2 Roche, J., *Biochem. J.*, 1931, **25**, 1724.

3 Roche, J., and Bullinger, E., *Enzymologia*, 1939, **7**, 278.

4 Folley, S. J., and Kay, H. D., *Ergebn. d. Enzymforsch.*, 1936, **5**, 159.

in laked red cell systems readily dephosphorylates monophenylphosphate,[2] the pH range of activity for the reaction showing an optimum at 5.8-6.0 but extending to well below pH 5.0.[2]

In estimating the "acid" phosphatase activity of blood serum[5, 6] by a recently described adaptation[7] of the King and Armstrong "alkaline" phosphatase method,[8] monophenylphosphate substrate is used with citrate buffer at pH 4.9. These conditions also permit of hydrolysis by such erythrocyte phosphatase as may be present if sufficiently hemolysed samples of blood serum are employed. The determination of serum "acid" phosphatases (which has certain clinical applications[9, 10, 11]) therefore gives misleading results in markedly hemolysed blood samples. If the use of such samples is unavoidable, a correction can be made after hydrolysis in the presence of NaF, which in proper concentration differentially inhibits serum "acid" phosphatases.

Experimental. Effect of hemolysis on phosphatase activity at pH 5.0. Fresh, non-hemolysed, normal sera exhibit slight "acid" phosphatase activity: 1.4 and 1.6 units/100 cc respectively in the 2 subjects cited in Table I. Defibrinated whole blood samples from these normal subjects showed more than 100 times greater phosphatase activity when determined at the same pH (5.0) by the same method, because large amounts of erythrocyte phosphatase are present in the hemolysates. Fresh, non-hemolysed sera of patients with metastasizing prostatic carcinoma contain prostate "acid" phosphatase and show elevated "acid" phosphatase levels (Table I). Defibrinated whole blood samples from these patients exhibit further increases in phosphatase activity at pH 5.0, due to the added effect of erythrocyte phosphatase.

If blood samples are only partially hemolysed and less erythrocyte phosphatase is present in the serum, less distortion of "acid" phosphatase values occurs. When ordinary precautions against hemolysis are taken, normal sera (even if slightly tinged) show no significant erythrocyte phosphatase activity.[5] Collection of blood with a wet syringe, however, results in high values: 14.7 units/100 cc in one

[5] Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 470.

[6] Gutman, A. B., and Gutman, E. B., *J. Clin. Invest.*, 1938, **17**, 473.

[7] Gutman, E. B., and Gutman, A. B., *J. Biol. Chem.*, 1940, **136**, 201.

[8] King, E. J., and Armstrong, A. R., *Canad. M. A. J.*, 1934, **31**, 376.

[9] Robinson, J. N., Gutman, E. B., and Gutman, A. B., *J. Urology*, 1939, **42**, 602.

[10] Gutman, A. B., Gutman, E. B., and Robinson, J. N., *Am. J. Cancer*, 1940, **38**, 103.

[11] Woodard, H. Q., and Higinbotham, N. L., *J.A.M.A.*, 1941, **116**, 1621.

TABLE I.
Phosphatase Activity at pH 5.0 of Fresh, Non-hemolysed Serum and of the Corresponding Whole Blood Hemolysate; Effect of 0.005 *M* NaF.
(0.005 *M* disodium phenylphosphate substrate, 0.1 *M* citrate buffer at 37°C. Results expressed in mg phenol liberated/100 cc/hr).

Source of blood	Serum	Serum + NaF	Inhibition by NaF %	Whole blood	Whole blood + NaF	Inhibition by NaF %
Normal subjects	1.4	0.1	93	210.	205.	2.4
	1.6	0.3	81	342.	338.	1.2
Cases of metastasizing prostatic carcinoma	56.0	7.2	87	259.	235.	9.3
	10.8	2.6	76	60.*	57.*	5.0
	60.0	7.2	88	172.**	169.**	1.7

* Partial hemolysis.
** This blood allowed to stand several days at room temperature. The prostate "acid" phosphatase has almost disappeared, the erythrocyte phosphatase present is little inhibited by NaF.

instance when the serum properly taken contained only 1.4 units. If such partially hemolysed samples of normal blood are allowed to stand several days, further increases occur. This does not apply to sera from patients with metastasizing prostatic carcinoma, as prostate "acid" phosphatase tends to disappear from the serum on prolonged standing at room temperature, in one case (after 72 hours) from 60.8 to 8.8 units/100 cc, despite some hemolysis.

Influence of different substrates and buffers. Monophenylphosphate appears to be the substrate of choice in the determination of serum "acid" phosphatase activity.[7] In hemolysed sera, however, the activity of erythrocyte phosphatase at pH 5.0 is more than 10 times greater with monophenylphosphate than with β-glycerophosphate substrate. As anticipated, marked distortion of serum "acid" phosphatase values was found also when α-glycerophosphate substrate was used in hemolysed sera. Erythrocyte phosphatase activity is approximately 20% greater with acetate than with citrate buffer at pH 5.0.

Effect of M/200 NaF. Most of the "acid" phosphatase activity of normal sera, and of patients with metastasizing prostatic carcinoma is inhibited by incubation in the presence of M/200 NaF. Erythrocyte phosphatase, however, is not significantly inhibited. In fresh but hemolysed sera, the presence of appreciable amounts of prostate "acid" phosphatase is indicated by a significant fall in phosphatase activity after incubation with NaF; the difference approximates the prostate "acid" phosphatase present.