TABLE II.

Dog No. / Sex		159 F	167 F	172 M	174 F
Avg B.P.	before first clamp	140 (4)*	130 (5)	120 (6)	135 (30)
" "	after " "	200 (4)	170 (39)	160 (43)	160 (20)
" "	" second "	—	205 (7)	195 (11)	—
" pH	before first "	7.30 (6)	7.34 (7)	7.33 (6)	7.38 (30)
" "	after " "	7.26 (3)	7.32 (39)	7.38 (45)	7.37 (22)
" "	" second "	—	7.31 (6)	7.37 (13)	—

*The figures in parentheses indicate the number of observations.

arterial blood pressure of 135 mm of mercury for the normal dogs, and a pH of 7.35 corresponding to an arterial pressure of 175 mm of mercury in the hypertensive dogs. This difference is within the experimental error of our method of measurement; however, since it is based on the average of many values, we feel that it may be significant enough to indicate the direction of pH changes in the blood due to clamping of the renal arteries. Further experiments are in progress to determine the pH changes in each renal vein.

With respect to Table II it may be mentioned that dogs 159 and 167 which had a sharp rise in arterial pressure with a change in blood pH towards more acid values, died before the experiment was finished due to uremia. The other hypertensive dogs with the more alkaline blood have a normal blood urea nitrogen content.

It may be concluded from this experiment that the buffering capacity of the systemic blood is sufficient to offset the introduction of an alkaline or acid substance if such is produced by the kidney as the result of ischemia.

11107

Bacteriostatic Actions of Three Thiazol Derivatives of Sulfanilamide upon Bacteria in Broth Cultures.

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On the basis of *in vitro* studies 3 members of a new group of compounds, namely 2-sulfanilamidothiazol (sulfathiazol), 2-sulfanilamido-4-methylthiazol (sulfamethylthiazol), both of which were recently described by Fosbinder and Walter,[1] and 2-sulfanilamido-4-

[1] Fosbinder, R. J., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2032.

phenylthiazol (sulfaphenylthiazol) appear to be distinctly superior to sulfanilamide and sulfapyridine in their bacteriostatic effects on pneumococci Types I, II and III, beta *Streptococcus hemolyticus* Group A, gonococci and *Staphylococcus aureus*.

Methods. Dilutions of the drugs were prepared in veal dextrose broth of pH 7.4 and containing bacto peptone. Sterile horse serum was added to the broth as an enriching substance for the pneumococci and streptococci. Ascitic fluid was used to enrich the medium for the gonococci.

To 5 cc of drug-broth solution was added one drop of an 18-hour undiluted broth culture of organisms. At the end of 5 hours' incubation at 37°C a transfer of 1 drop was made from the first tube to a second containing the same drug-broth solution, as well as to a tube containing no drug. Similar transfers were made from the second to the third tube, from the third to the fourth tube, etc., at the end of 8, 24, 32, 48, 56, and 72 hours. All tubes were incubated at 37°C for at least 96 hours before recording the final results. A protocol on the findings of one of these tests is presented in Table I.

Results. After 5 hours' exposure of one drop of Types I, II and III pneumococci in a 5 mg % concentration (1:20,000) of the thiazol-broth solutions, a drop of the treated organism suspensions failed to elicit a growth in a second tube containing the same drug-broth solution. Sulfapyridine, on the other hand, did not exhibit this same action on Types II and III even after the seventh transfer or at the end of 72 hours. The latter compound showed some degree of bacteriostasis upon Type I pneumococci, however, here again not to the extent demonstrated by the thiazol derivatives. The sulfaphenyl

TABLE I.

Bacteriostatic Action of a 5 mg % Concentration of Sulfanilamide and Some of Its Derivatives upon Type II Pneumococci in Broth.

Organism	Compound	Time intervals of transfers in hrs. 0-5	5	8	24	32	48	56	72
Type II Pneumococci	Sulfanilamide	+	+	+	+	+	+	+	+
	Control		+	+	+	+	+	+	+
	Sulfapyridine	+	+	+	+	+	+	+	+
	Control		+	+	+	+	+	+	+
	Sulfathiazol	+	—	—	—	—	—	—	—
	Control		+	+	+	—	—	—	—
	Sulfamethylthiazol	+	—	—	—	—	—	—	—
	Control		+	+	+	+	—	—	—
	Sulfaphenylthiazol	+	—	—	—	—	—	—	—
	Control		+	+	—	—	—	—	—
	Broth culture control	+	+	+	+	+	+	+	+

+ Indicates growth.
— Indicates no growth.

Fig 1.

Bacteriostatic Actions of Sulfapyridine and Sulfanilamidothiazol Compounds upon Pneumococci and Streptococci

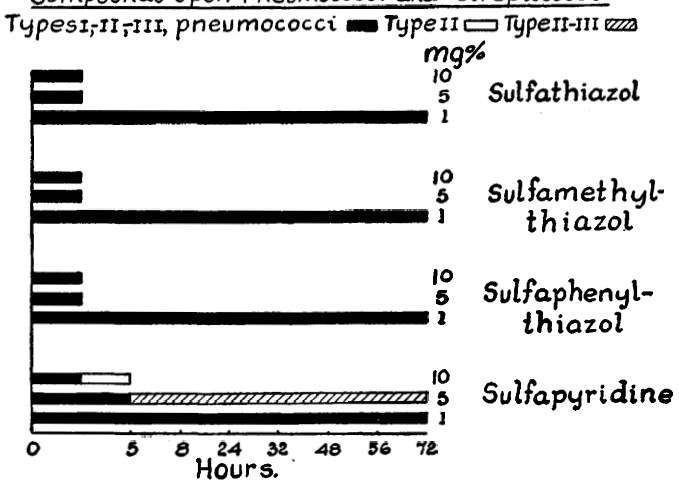
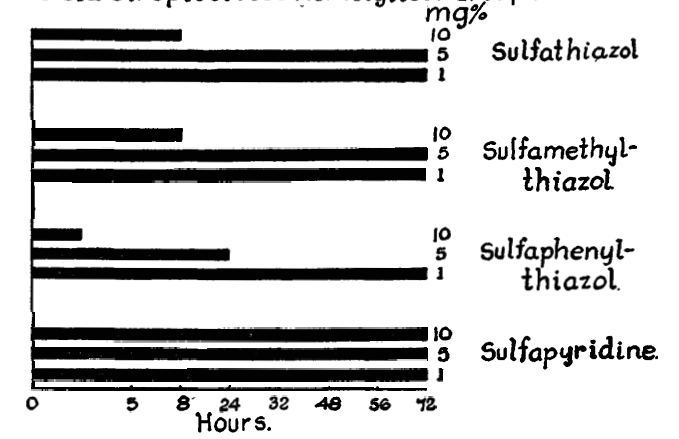


Fig 2.

Beta Streptococcus Hemolyticus Group "A"



The length of the columns for time intervals less than 72 hours indicates the time at which complete bacteriostasis occurred in the drug-broth tubes. Columns extending to 72 hours indicate growth up to this time.

compound was the only drug which presented inhibition upon the streptococci in this low concentration.

A 10 mg % concentration (1:10,000) of sulfathiazol and sulfamethylthiazol inhibited the development of streptococci in the third transfer or in the 24-hour subculture tube. The phenyl derivative was correspondingly effective in less than 5 hours; in other words, no growth was detected beyond the first inoculated tube. Sulfapyridine

produced no demonstrable effect in the same concentration throughout the entire test period. A summary of the results on pneumococci Types I, II and III and beta *Streptococcus hemolyticus,* using 1, 5, and 10 mg % concentrations of the drugs are presented in Figs. 1 and 2. The results obtained with sulfanilamide are omitted in the figures, since it was found that a 10 mg % concentration of the compound failed to produce total inhibition of any of the test organisms.

A 1 mg % concentration (1:100,000) of the 3 thiazol compounds inhibited the development of the gonococcus in the first subculture tubes, although proliferation was noted to have occurred in the first inoculated tube. Sulfapyridine in the same test inhibited growth of the organisms only at the 56-hour transfer. Sulfanilamide was ineffective throughout the entire test period. The results of this test are presented in Table II.

The action of all compounds which were effective under the conditions of these tests has been found to be purely one of bacteriostasis. These findings confirm the results obtained with the parent substance as reported by Bliss and Long,[2] Gay and his coworkers,[3] and others. By preparing dilute suspensions of the various organisms in saline and making agar plate counts, the bacteria in all instances were found to have multiplied in the first tube inoculated with the heavy broth suspension. Total inhibition, when such did occur, was first noted in the drug-broth subculture tubes. Furthermore, when bacteriostasis in the latter tubes became apparent, the corresponding control tubes containing no drug always yielded a growth in at least

TABLE II

In Vitro Effects of a 1 mg % Concentration of Sulfanilamide and Some of Its Derivatives upon Gonococci in 20% Ascitic Fluid Broth.

Organism	Compound	Time intervals of transfers in hrs.							
		0-5	5	8	24	32	48	56	72
Gonococcus Strain No. 66	Sulfanilamide	+	+	+	+	+	+	+	+
	Control		+	+	+	+	+	+	+
	Sulfapyridine	+	+	+	+	+	+	—	—
	Control		+	+	+	+	+	+	—
	Sulfathiazol	+	—	—	—	—	—	—	—
	Control		+	—	—	—	—	—	—
	Sulfamethylthiazol	+	—	—	—	—	—	—	—
	Control		+	+	—	—	—	—	—
	Sulfaphenylthiazol	+	—	—	—	—	—	—	—
	Control		+	+	—	—	—	—	—
	Broth culture control	+	+	+	+	+	+	+	+

\+ Indicates growth.
— Indicates no growth.

[2] Bliss, E. A., and Long, P. H., *J. A. M. A.*, 1937, **109**, 1524.

[3] Gay, Clark, Street and Miles, *J. Exp. Med.*, 1939, **69**, 607.

one transfer beyond the point at which inhibition was detected in the drug-broth solutions.

Since it was found that a 10 mg % concentration of the various compounds had little if any effect in inhibiting the growth of *Staphylococcus aureus* under the above conditions, a second method of testing these compounds on this organism was resorted to. Accurately weighed quantities of the drugs were added to 100 cc portions of veal dextrose broth. Due to the poor solubility of some of the compounds the solutions tested were, in certain instances as indicated in Table III, supersaturated. To each drug-broth solution, or solution-suspension was then added 1 cc of a dilute suspension of *Staphylococcus aureus*. All flasks containing the control and medicated media were maintained at 37°C by means of a water bath. Immediately following the addition of the inoculum, and hourly intervals thereafter, 0.1 cc of the organism-broth suspension was transferred to the center of a sterile petri dish. Melted and cooled (45°C) veal dextrose agar was then added to the plate, the contents mixed thoroughly by swirling and the agar allowed to solidify. The plates were then placed in the incubator at 37°C for 72 hours, at which time the results were recorded.

The data presented in Table III indicate that the thiazol compounds, and especially the methyl and phenyl derivatives exhibit a marked bacteriostatic action upon *Staphylococcus aureus*. Furthermore, although not indicated in the table, this inhibitory effect was conspicuous even after several days' incubation at which time interval little, if any cloudiness, indicative of growth could be detected

TABLE III.
Evaluation of Bacteriostatic Action of Sulfanilamide and Some of Its Derivatives upon *Staphylococcus aureus* by Direct Plate Counts.

Compounds	mg %	Solution	Hours						
			0	2	3	4	5	6	7
Sulfanilamide	200	Complete	11	26	70	221	387	950	1,800
	100	"	5	34	149	441	930	1,500	3,000
Sulfapyridine	200	Incomplete	13	19	45	186	349	800	1,500
	100	Complete	14	23	93	340	620	950	2,500
Sulfathiazol	200	"	10	9	32	95	183	364	660
	100	"	12	16	58	170	339	900	1,000
Sulfamethylthiazol	200	Incomplete	4	9	14	29	52	107	196
	100	Complete	10	17	49	133	287	760	1,400
Sulfaphenylthiazol	100	Incomplete	7	6	12	11	12	34	37
	50	"	10	13	15	19	19	32	47
	10	Complete	10	16	50	134	229	720	990
Broth Control	—	—	9	27	124	500	1,200	8,500	*

Figures of 500 or less represent actual number of colonies counted per plate. Values above 500 were approximated on the basis of counts of uniform fields.
*Too numerous to count.

in the inoculated flasks containing the larger quantities of the latter two compounds. Under similar conditions sulfanilamide and sulfapyridine exhibited a moderate bacteriostatic action but these compounds were distinctly inferior to the thiazol derivatives against the staphylococcus in the drug concentrations used. Under different experimental conditions Bliss and Long[4] reported that sulfanilamide and sulfapyridine were ineffective in their *in vitro* action upon *Staphylococcus aureus.*

Summary. Three new thiazol derivatives of sulfanilamide have been studied with respect to their bacteriostatic action upon microorganisms. These compounds were found to be superior to sulfanilamide and sulfapyridine in their inhibitory actions upon pneumococci Types I, II and III and beta *Streptococcus hemolyticus* Group A in concentrations as low as 5 mg %. Concentrations of 1 mg % proved the new derivatives to be more effective against the gonococcus than the parent compound and sulfapyridine. The methyl and phenyl derivatives were found to be markedly bacteriostatic for *Staphylococcus aureus.* Sulfanilamide and sulfapyridine exhibited a moderate degree of inhibition upon the latter organism.

11108

Negative Effect of Synthetic Vitamin B_6 Hydrochloride in Nutritional Deficiency in Man.*

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(Introduced by George R. Minot.)

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Spies and his associates[1] have recently reported observations on 4 patients with pellagra, who following treatment with nicotinic acid, riboflavin and thiamin chloride while they were taking a deficient diet, continued to complain of nervousness, insomnia, irritability, abdominal pain, weakness and difficulty in walking. These symptoms were relieved within 4 hours by a single dose of 50 mg of synthetic vitamin B_6 hydrochloride (2-methyl-3-hydroxy-4, 5-(hydroxymethyl)-pyri-

[4] Bliss, E. A., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 483.

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[1] Spies, T. C., Bean, W. B., and Ashe, W. F., *J. Am. Med. Assn.*, 1939, **112**, 2414.