Thus, 650 mice have been used in tests of the efficacy of the drugs when administered by methods other than that of addition to the diet. Approximately 1200 mice have been used in experiments with a 1% diet and 200 on a 0.5% diet.

As has been shown in Table I both sulfathiazole and sulfapyridine have an appreciable therapeutic effect against the agent of *lymphogranuloma venereum*. In tests with this agent 85 mice have been used. These results are in agreement with those obtained by others with sulfanilamide. Neither sulfathiazole nor sulfapyridine, however, has shown any activity against swine-influenza virus or *herpes simplex*. In these tests 300 mice have been used. Further virus-studies are in progress.

Conclusions. It has been shown that when the compounds which have been studied are administered as 1% of the diet, the therapeutic effect of sulfathiazole is equal to that of sulfapyridine. In view of the comparative toxicity and metabolism of these two compounds[2] it is possible that sulfathiazole may be a more desirable therapeutic agent than sulfapyridine.

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Chemotherapeutic Evaluation of Sulfanilamide Derivatives of Heterocyclic Amines.

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The therapeutic success with which sulfanilamide has been used in a number of unrelated bacterial and several viral diseases has stimulated the search for more effective and less toxic drugs. The trend of these investigations has generally been to modify the sulfanilamide nucleus by substitution in the amine or amide group, replacement of the amide group or degradation of the sulfone group. These substitutions have generally been of the alkyl, aryl, or substituted-alkyl or substituted-aryl type. The few compounds formed by the substitution of heterocyclic radicals for amino or amide hydrogens were of no especial interest, prior to the advent of sulfapyridine, because of their relatively feeble therapeutic activity.

Of these, the heterocyclic azo compounds *p*-sulfonamidophenylazodihydrocupreine,[1, 2] *p*-sulfonamidophenylazoapoquinine,[2] *p*-sulfonam-

[1] Heidelberger, M., and Jacobs, W. A., *J. Am. Chem. Soc.*, 1919, **41**, 2145.

[2] Buttle, G. A. H., Gray, W. H., and Stephenson, D., *Lancet*, 1936, **1**, 1286.

idophenylazoisoapoquinine,[2] and *p*-sulfonamidophenylazoisoquinidine[2]and also tropinone bis-sulfonamideophenylhydrazone[2] possessed very litle therapeutic activity.

The amino substituted compounds *p*-furfurylideneaminophenylsulfonamide[3] and 2-pyrrolidone-5-carboxy-4′-aminophenylsulfonamide[3] were claimed to be respectively less active and more active than sulfanilamide in streptococcic infections.

The two nitrogen-linked sulfones, l-sulfanilylpiperidine[3] and l-sulfanilylpiperazine* were inactive, whereas 2-pyrrolidone-5-carboxy-4′-amino-phenylsulfinic acid[4] was reported to be as active as sulfanilamide.

The amide substituted compounds, *p*-aminophenylpiperidylsulfonamide[5] and 2-sulfanilamidopyridine (sulfapyridine)[6] were claimed to be respectively one-half as active and as good as or better than sulfanilamide against streptococcic infections. The latter compound, in adequate dosage, was claimed by Whitby[6] to save all mice infected intraperitoneally with 10,000 fatal doses of Types I, VII, and VIII pneumococci.

Subsequent investigations have shown sulfapyridine to be fractionally superior to sulfanilamide against mouse infections of approximately 10 to 1000 fatal doses of pneumococci[7-11] and of almost equal efficacy against experimental pneumococcus pneumonia[11] and meningitis[7, 11] of rats.

Even though the demonstrated superiority of sulfapyridine over sulfanilamide when used in equal doses in experimental infections, was considerably less than that originally claimed for it, this slight improvement suggested the possibility that more effective therapeutic agents might result from other heterocyclic substitutions of sulfanilamide. Accordingly, the following 5 compounds,† which contain component nuclei characteristic of other well known medicinals,

[3] Gray, W. H., Buttle, G. A. H., and Stephenson, D., *Biochem. J.*, 1937, **31**, 724.

* Synthesized and donated by the Monsanto Chemical Company, St. Louis, Mo.

[4] Buttle, G. A. H., Dewing, T., Foster, G. E., Gray, W. H., Smith, S., and Stephenson, D., *Biochem. J.*, 1938, **32**, 1101.

[5] Poulain, P., *These de Paris*, 1936.

[6] Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

[7] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 37.

[8] Hilles, C., and Schmidt, L. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 73.

[9] Long, P. H., Bliss, E. A., and Feinstone, W. H., *Pennsylvania M. J.*, 1939, **42**, 483.

[10] Schmidt, L. H., and Hilles, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 611.

[11] Cooper, F. B., Gross, P., and Lewis, M., *J. Clin. Invest.*, 1939, **18**, 423.

† Synthesized and donated by the Maltbie Chemical Company, Newark, N. J.

TABLE I.
Streptococcic Infection of Mice (1,000-10,000 Fatal Doses).

	Treatment	No. of Mice	No. of deaths daily during 3 weeks									No. of Survivors	% of Survivors
			1	2	3	4	5	6	7	8	9-21		
None		50	32	7	3	1	1	1	2	2	1	0	0
I	Sulfanilamide	50		4		1		1	1		5	38	76
II	Sulfapyridine	30			1		1					28	93
III	2,6-Diamino-3-p-sulfonamidophenylazopyridine	10	3			2		1			2	2	20
IV	2-N4-acetylsulfanilamido-6-aminopyridine	10				2			2			6	60
V	2-Sulfanilamido-6-aminopyridine	40	4	1	2			1	1		3	28	70
VI	2-Sulfanilamidothiazole	30	1	1	1		1	1			2	23	77
VII	2-Sulfanilamido-4-methylthiazole	30	1			1			1		2	25	83

Infection: 0.5 cc of a 10^{-4} broth dilution of an 18-hour broth culture (C 203) intraperitoneally (1,000-10,000 fatal doses).
Treatment: 10 mg of drug suspended in 0.2 cc of 15% gum acacia orally 3 hours after infection, then once daily for 3 successive days (total 40 mg).

TABLE II.
Streptococcic Infection of Mice (100,000-1,000,000 Fatal Doses).

	Treatment	No. of Mice	No. of deaths daily during 3 weeks									No. of Survivors	% of Survivors
			1	2	3	4	5	6	7	8	9-21		
None		25	25									0	0
I	Sulfanilamide	25	20	2							1	2	8
II	Sulfapyridine	25	13	2	1							9	36
V	2-Sulfanilamido-6-aminopyridine	25	19							1	1	4	16
VI	2-Sulfanilamidothiazole	25	18						2			5	20
VII	2-Sulfanilamido-4-methylthiazole	25	17	1						1	1	5	20

Infection: 0.5 cc of a 10^{-2} broth dilution of an 18-hour broth culture (C 203) intraperitoneally (100,000 to 1,000,000 fatal doses).
Treatment: 10 mg of drug suspended in 0.2 cc of gum acacia orally 3 hours after infection, then once daily for 3 successive days (total 40 mg).

TABLE III.
Type II Pneumococcic Infection of Mice (100 Fatal Doses).

	Treatment	No. of Mice	No. of deaths daily during 3 weeks									No. of Survivors	% of Survivors
			1	2	3	4	5	6	7	8	9-21		
None		30	2	10	13	4	1					0	0
I	Sulfanilamide	30		1		1	3	3	7	1	2	12	40
II	Sulfapyridine	30			1		1		4	2	4	18	60
III	2,6-Diamino-3-p-sulfonamidophenylazopyridine	10	6	1	3							0	0
IV	2-N^4-acetylsulfanilamido-6-aminopyridine	10	1	3	4	2						0	0
V	2-Sulfanilamido-6-aminopyridine	30	4	4		1				3	3	15	50
VI	2-Sulfanilamidothiazole	30	1	1			5		1	6	3	13	43
VII	2-Sulfanilamido-4-methylthiazole	30			1			2	4	3	6	14	47

Infection: 0.5 cc of a 10^{-6} broth dilution of an 18-hour broth culture of Type II (Binda) subcutaneously (100 fatal doses).

Treatment: 20 mg of drug suspended in 0.2 cc of 15% gum acacia orally 3 hours after infection, then once daily for 5 successive days (total 120 mg).

were synthesized[12] and their antistreptococcic and antipneumococcic activities compared to those of sulfanilamide and sulfapyridine: 2,6-diamino-3-p-sulfonamidophenylazopyridine; 2-N^4-acetylsulfanilamido-6-aminopyridine; 2-sulfanilamido-6-aminopyridine; 2-sulfanilamidothiazole; 2-sulfanilamido-4-methylthiazole.

The therapeutic activities of these compounds were determined by inoculating mice with a number of infecting doses just within or slightly beyond the effective range of sulfanilamide and sulfapyridine therapy and maintaining the treatment somewhat below the optimum level. In this manner, differences in drug efficacy are more readily seen than when all animals in two or more treated groups survive.[13] Thus, the values obtained do not indicate the maximum number of survivors which would have been obtained under optimum treatment but rather the relative values of the various drugs over an adequate period of observation.

Tables I, II, and III show sulfapyridine to be somewhat superior to compounds V, VI,‡ and VII,‡ which possess approximately the same degree of therapeutic activity as sulfanilamide, against streptococcic and Type II pneumococcic infection in mice.

Main, Shinn and Mellon[14] have reported that bacteriostasis and the accompanying increased accumulation of hydrogen peroxide per unit of growth were similar in cultures of Type I pneumococcus which contained comparable amounts of sulfanilamide, sulfapyridine, VI or VII.

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Associative Hysteresis in Larval Amblystoma.

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The object of the study was to determine whether larval amblystoma are able to establish associations with non-essential signals, *i. e.*, are capable of true learning. This necessarily rules out such effects of training as facilitation of primary reflexes. It was found

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

[13] Cooper, F. B., Gross, P., and Lewis, M., *J. Chemotherapy*, 1938, **15**, 31.

‡ These drugs, like sulfapyridine, produce renal concrements in rats. A detailed study of these, and other toxicological data will be published shortly.

[14] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 115.