Summary. A new Salmonella type, *S. lomalinda,* was described. The culture was represented by the antigenic formula IX,XII: a-e,n,x . . . This formula was erroneously attributed to *S. durban,* which in reality possesses antigens IX,XII:a-e,n,z_{15} . . . The two types presented biochemical as well as antigenic differences.

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Tuberculostatic Action of Phenothiazine and Derivatives.*

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Phenothiazine has been proposed as an insecticide, fungicide, anthelmintic, and urinary antiseptic.[1,2] Many phenothiazine derivatives, such as methylene blue, Lauth's violet, phenothiazone, and thionol form oxidation-reduction systems. Phenothiazine, after absorption in the body, is excreted partly in the oxidized forms of phenothiazone and thionol, as well as in their respective leuko forms, with which they form oxidation-reduction systems.[3] Exposure of phenothiazine to air and light results in oxidation to phenothiazone and possibly to thionol, in which forms they become more active as fungicides. In the following experiments this interesting group of compounds was tested for bacteriostatic effect on the growth of tubercle bacilli *in vitro.*

I. The bacteriostatic action of phenothiazine was tested on the highly virulent strain of tubercle bacilli, H37Rv. Proskauer and Beck liquid synthetic asparagin media, pH 7.2, was used. Phenothiazine was dissolved in propylene glycol to make a 1% solution. Subsequent dilutions were made directly into the media. Appearance of a pellicle at the end of 3 weeks was taken as the criterion of growth. The highest dilution at which bacteriostatic action occurred was 1:1,000,000.

II. Phenothiazine and 15 derivatives were tested for tuberculostatic action on an avirulent Novy strain of tubercle bacilli. This culture normally produces a heavy surface growth within 4 days. Most of the chemical compounds were dissolved in propylene glycol. Methylene blue and Lauth's violet were dissolved in distilled water. The compounds and the results are listed in Table I. The parent compound, phenothiazine, again inhibited growth in concentration of 1:1 million. Phenothiazine sulfate also showed a high bacteriostatic effect in concentration of 1:200,000. The oxidized derivatives, phenothiazone and thionol (Nos. 3 and 4), showed a moderate degree of inhibition, in dilutions of 1:100,000 and 1:80,000 respectively. The *x*-hydroxyphenothiazine, apparently in the oxidized form in solution, gave results comparable with its isomer, phenothiazone. It is of interest that these oxidized forms are less effective than phenothiazine, as the reverse is the case in their inhibiting action on certain fungi. This suggests that either the oxidized forms are less readily absorbed by the tubercle bacilli, or the interference with the metabolism of the tubercle bacilli is of a different type than that of its fungicidal action. The oxidized forms are less soluble in lipids than phenothiazine; this may decrease the ability of these compounds to penetrate the tubercle bacilli in sufficient concentration.

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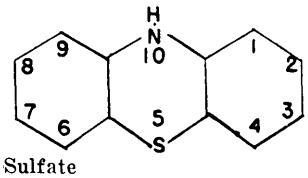
1 DeEds, F., Stockton, A. B., and Thomas, J. O., *J. Pharm. and Exp. Therap.,* 1939, **65**, 353.

2 Prelim. Report, Council of Pharm. and Chem., *J. A. M. A.,* 1940, **115**, 1721.

3 DeEds, F., Wilson, R. H., and Thomas, J. O., *J. Pharm. and Exp. Therap., J. A. M. A.,* 1940, **114**, 2095.

TABLE I.
Tuberculostatic Action.

No.	Compound	Concentration, 1 part in:
1	Phenothiazine 	1,000,000
2	Phenothiazine Sulfate	200,000
3	Phenothiazone (3-oxyphenothiazine)	100,000
4	Thionol (3-oxy,7-hydroxyphenothiazine)	80,000
5	x-Hydroxyphenothiazine	80,000
6	Methylene Blue (3,7-tetramethyldiaminophenothiazine)	<10,000
7	Lauth's Violet (3,7-diaminophenothiazine)	20,000
8	N-Benzoylphenothiazine	<10,000
9	N-Methylphenothiazine	<10,000
10	5,10-Dimethyl Phenothiazonium,5-methosulfate	10,000
11	Benzo(a)phenothiazine	<10,000
12	Benzo(c)phenothiazine	10,000
13	x-Cyclohexylphenothiazine	<10,000
14	N-Nitrosophenothiazine	20,000
15	Chlorinated Phenothiazine*	<10,000
16	Dodecahydrophenothiazine	<10,000

Most of the compounds were obtained through the courtesy of the Dow Chemical Co., Midland, Mich. Phenothiazone and thionol were kindly provided by Dr. F. DeEds, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Albany, Calif.
* One or more chlorine atoms.

The thiazine dyes, methylene blue, and Lauth's violet, showed relatively poor inhibition, as did also the remainder of the compounds. Substitution of the hydrogen attached to the nitrogen of phenothiazine by methyl, benzoyl, or nitroso groups destroyed the bacteriostatic effect of phenothiazine. In order to be effective it may be necessary that this hydrogen remain unsubstituted, to permit the compound to form oxidation-reduction systems with its leuko form.

All the compounds were tested at pH of 6.4 and 7.7. No significant differences in the tuberculostatic action of any of the compounds was noted. Phenothiazine, phenothiazone, and thionol were tested in the presence of 20% horse serum. Phenothiazine showed inhibition in concentration of 1:40,000; phenothiazone and thionol in concentration of 1:20,000. Experiments are in progress to determine the therapeutic effect of these compounds in animals.

Summary. Phenothiazine inhibited the growth of tubercle bacilli *in vitro* in high dilution. In the presence of serum the bacteriostatic effect was diminished, but was still significant. Oxidized forms of phenothiazine showed moderate inhibition of growth. Other derivatives of phenothiazine exhibited low tuberculostatic action.