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TUBERCULOCIDAL ACTION OF CERTAIN CHEMICAL DISINFECTANTS *

STUDIES OF THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS, IX

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In all the great mass of literature on general disinfection, we have been able to find but little dealing at all directly with the power of chemicals to kill tubercle bacilli. It has been generally accepted that tubercle bacilli, though non-sporogenous, are among the most resistant of pathogenic organisms.

Drying-out processes seem to have little effect on either their vitality or their pathogenicity. Authors have differed regarding the action of dry heat, but Krumwiede,[1] in carefully controlled experiments, found that tubercle bacilli dried for five days and subjected to dry heat at 100 C. were killed in one hour and fifteen minutes so that inoculated animals failed to develop the disease. Some tubes were killed in twenty minutes. Theobald Smith[2] noted that cultures of tubercle bacilli, which had been kept from six weeks to two months, would not grow when transferred to fresh tubes of glycerin agar. Supposing such cultures to be dead, he used them for immunization experiments on guinea-pigs and found that cultures, which had been kept from seven to twenty months,

* Received for publication May 11, 1914.
1. *Rept. Research Lab. Dept. of Health,* New York City, 1913.
2. *Jour. Med. Research,* 1913, 28, p. 91.

still contained a sufficient number of living bacilli to confer the disease upon susceptible animals. The earliest report found of chemical disinfection of tubercle bacilli was published in 1888 by Yersin.[3] Earlier experiments on the disinfection of tuberculous sputum had been published, but as these do not permit of scientific accuracy and control, they will be omitted and we will review only those dealing with the action of chemicals on pure cultures of tubercle bacilli. Yersin used pure cultures fifteen days old. He exposed small bits to the action of his disinfectants for variable times, then dropped the bits into distilled water. After several hours in the sterile distilled water, the well-washed bit of culture was transferred to flasks of glycerin broth and incubated at 39 C. If no growth developed, he concluded that the organisms were killed. Thus he states that tubercle bacilli exposed for 30 seconds to 5 percent phenol, or one minute to 1 percent, were killed. Absolute alcohol killed in five minutes, iodoformed ether, 1 percent, killed in 5 minutes, while pure ether required 10 minutes, and 0.3 percent thymol destroyed the organisms in two hours. This report is interesting historically, since it is one of the earliest disinfectant reports. It is also interesting because it is the only investigation which we have found in the literature in which any systematic study of the action of chemical disinfectants on tubercle bacilli has been made. It is unfortunate that the author confined his tests, on the death of the organism, entirely to artificial cultures, and inoculated no animals with the treated bacilli, since, as Theobald Smith stated, the animal body is the best culture media for the growth of tubercle bacilli. It is also unfortunate that he did not realize that while the organisms were being washed in distilled water, they were under the influence of the diluted disinfectant, since, if we understand him correctly, the water was not changed, nor was any effort made to neutralize the disinfectant. It can scarcely be doubted, therefore, in the light of more recent disinfectant work, that the times reported in his experiments must inevitably be too short.† Practically all the other reports found by us have been either on the disinfection of sputum and other excreta or brief mention of tubercle bacilli used with many other bacteria in the larger works on the testing of disinfectants. Thus Bechhold,[4] in testing halogen derivatives of phenol and naphthol groups, found that they had a much stronger action than lysol on the staphylococcus aureus, streptococcus, the bacilli coli, typhosus, diphtheriae, etc. On human tubercle bacilli, however, they had no effect; a 2.5 percent solution of tri-tetra-brom β Naphthol failed to kill these organisms in twenty-five hours, although 5 percent lysol containing 2.5 percent cresol killed the tubercle bacilli in four and one-half to eight hours. In Bechold's experiments, emulsions of human tubercle bacilli were exposed to equal volumes of the disinfectant, and then injected into animals. No attempt was made to remove the disinfectant or to check its action, as the disinfectant and organisms were injected together. Inhibition may therefore play some part, although the long time required for the disinfectant action of lysol suggests that its part cannot have been very great. Green[5] found that chloroform vapor mixed with air and passed through tubes containing vaccine lymph and different kinds of bacteria, including tubercle bacilli, sterilized the lymph so that no growth developed when it was inoculated

3. *Ann. de l'Inst. Pasteur,* 1888, 2, p. 60.

† At the time at which Yersin worked slight attention was paid to the strain of tubercle bacillus used. Discrepancies between the results of the French and the German workers led to an investigation of this point, and it was found that the French workers were dealing with bovine, while the German bacteriologists were studying human, tubercle bacilli. So Yersin's work was done with pure cultures of tubercle bacilli, but they were undoubtedly of the bovine type.

4. *Ztschr. f. Hyg. u. Infectionskrankh.,* 1909, 64, p. 113.

5. *Lancet,* 1904, 1, p. 1498.

on suitable media. Cantani[6] states that iodin in dilutions of 1 to 500, or even 1 to 1,000, had considerable influence on tubercle bacilli. Benians[7] found that emulsions of tubercle bacilli shaken for fifty minutes, or for twenty-four hours with 10 percent toluol, when injected into guinea-pigs caused no infection. Wells and Corper[8] in a similar way found that tubercle bacilli exposed for twenty hours, and for five days to toluol water, and then injected into guinea-pigs, in no case caused tuberculosis. Lumière and Chevrotier[9] investigated a large number of metallic salts with reference to their inhibitory action on tubercle bacilli. Cadmium chlorid and mercuric chlorid were the most active in inhibiting growth, 1 in 4,000 being sufficient for that purpose. Rosenthal[10] finds that gold cyanid in dilution of 1 to 2,000,000 inhibits the growth of tubercle bacilli, but he admits that the action is inhibitory, as he obtains a luxuriant growth if he washes the organisms after seventy-two hours' exposure to dilute gold cyanid solutions. Biasiotti[11] states that colloidal silver in high dilutions kills the staphylococcus aureus, the bacillus typhosus and diphtheriae, and, undiluted, kills the spores of the bacillus anthracis, but not tubercle bacilli. May[12] found that basic fuchsin had some germicidal action, killing the bacilli typhosus, paratyphosus, coli, and dysenteriae in five minutes. He also found that the staphylococcus aureus and the saprophytic form of the tubercle bacillus could be killed by this dye. DeWitt[13] has shown that methylene blue, in high dilution, inhibits the growth of tubercle bacilli, and while not all the organisms are killed, the vitality of the culture is so lowered that the disease develops slowly, frequently causing a local process only. Mercury salts or trypan-blue also showed marked tuberculocidal properties, only one out of the six animals used developing the disease.

Since, then, so little has been systematically done on the chemical disinfection of the tubercle bacillus, it has seemed advisable to test a number of the common disinfectants in order to determine their action on human tubercle bacilli. It is probable that the small amount of work on this problem is due less to lack of appreciation of its importance than to the difficulties that one encounters in finding a method, which is at the same time accurate and not too time-consuming and complicated.

At the beginning of our investigations, we tried numerous methods — the silk-thread method of Koch, the garnet method of Krönig and Paul, and emulsion methods. We early found that no emulsion method could be depended on to give uniform results on culture tubes. Some tubes would show growth, which was usually delayed, but the breaking up of clumps and separation of the organisms from each other seemed sufficient to prevent or greatly delay growth on agar tubes

6. *Ztschr. f. Hyg. u. Infectionskrankh.*, 1909, 63, p. 34.
7. *Ztschr. f. Chemoth.*, 1913, Orig., 11, p. 28.
8. *Jour. Infect. Dis.*, 1912, 11, p. 388.
9. *Bull. gén. de thérap.*, 1913, 165, p. 959.
10. *Ibid.*, p. 961.
11. *Ann. d'ig. sper.*, 1909, 19, p. 543; Reviewed in *Centralbl. f. Bakteriol.*, 1910, Ref., 45, p. 680.
12. *Jour. Am. Med. Assn.*, 1912, 48, p. 1174.
13. *Jour. Infect. Dis.*, 1913, 12, p. 68, and 13, p. 378.

or even in glycerin broth, even without exposure to any disinfectant. In other words, single organisms, separated from their companions, lack the vitality necessary for uniformly successful culture on artificial media. It seemed necessary, therefore, to expose a clump of the bacteria to the action of the disinfectant. The time of exposure in nearly all experiments was one hour, six hours, and twenty-four hours. As we desired to control the time sharply and to remove inhibiting factors as completely as possible, the clumps were placed, at the end of the specified time, either in a neutralizing fluid or in water. In all cases, the clumps were washed through four solutions, the last two being 0.9 percent salt solutions. The cultures used were, in the main, young, generally from three to four weeks old, and in all cases human tubercle bacilli. As the clumps showed a tendency to break up in the fluids so that it was difficult and time-consuming to handle them, and contaminations frequently occurred, several methods of confining the clumps were tried, a gauze bag method being finally adopted. Draw-strings were run into a small square of gauze so that, after placing the clumps of tubercle bacilli on it, it could be drawn up into a small pouch. These gauze bags were, of course, thoroughly sterilized in Petri dishes before use. At the beginning of the experiment, clumps of culture of approximately equal size were placed in the bags, which were drawn up tightly and placed in the disinfectant solution, of which a uniform, large amount was always used. At the end of the desired time the bag was removed with sterile platinum forceps and thoroughly washed as before stated. It was then opened and the contents floated out into a small tube of sterile salt solution. The bits of culture were then seeded on slants of 2 percent glycerin agar, 0.8 percent acid to phenolphthalein. As we realized that objection might readily be raised against this method, on account of the difficulty of penetration of the disinfectant into the clumps, and especially on account of the difficulty of equal penetration of all clumps, since some of course broke up more readily than others, our results by this cruder method of culture tubes were in all cases controlled with animal cultures. For these we used the garnet method suggested by Krönig and Paul.[14] Garnets of approximately equal size were soaked in a thin filtered suspension of human tubercle bacilli such that very few, if any, bacilli were present in clumps. They were then dried over $CaCl_2$. About thirty infected

14. *Ztschr. f. Hyg. u. Infectionskrankh.*, 1897, 25, p. 1.

garnets were then placed on small platinum baskets and immersed in the various disinfectant dilutions, of which 50 c.c. were allowed for each basket. At the end of the desired times, the baskets were removed to dishes containing large quantities of distilled water, then either to ammonium sulphid or to another dish of distilled water, and then washed in two salt solutions. Ten of the treated and washed garnets were then dropped into test tubes each containing 2 c.c. of sterile salt solution and shaken in a shaking machine for five minutes. The fluid containing the organisms, which had been shaken from the garnets, was then injected subcutaneously into guinea-pigs. For each time and each dilution four tube cultures and three guinea-pigs were used, a sufficient number, it was believed, to admit of a trustworthy judgment being made from consistent results, whether positive or negative. For controls, in each experiment, clumps and garnets were treated in the same way, 0.9 percent salt solution being used in place of the disinfectant, and both tube and animal cultures being made. It may be stated here, that in not a single case out of all the series used did a control guinea-pig fail to develop local and general tuberculosis, nor a control tube culture fail to show luxuriant growth. It may, therefore, fairly be assumed that lack of growth in the four tubes and the three animals may be ascribed to a bactericidal influence of the disinfectant used. With nearly all the disinfectants the experiment was repeated two to four times with the tube cultures, and when the first animal experiment gave non-uniform results, that too was repeated. Altogether approximately one thousand guinea-pig cultures and many more tube cultures have been employed to establish our results, which are given, in brief, in Table 1. The minimum time of one hour was chosen for convenience, but with phenol a five-minute interval was also employed in the animal experiments and found to give approximately the same results. In Table 1, T. is used for tube culture; A for animal culture; percentage dilutions are indicated at the head of the columns; + indicates a growth of the culture in all four tubes; $\pm$ or $\mp$ indicates variation in results, the upper sign suggesting which predominates. In the animal column two signs are used, the first for local tubercle and the second for general involvement. Thus + + means both local and general tuberculosis, while + — means local processes but no general. The $\pm$ and $\mp$ signs are used in the same way as was explained for the tube cultures.

The test tube cultures were kept two months or more before the final report was made, although the control tubes were well developed in one month or less. The animals were not considered as positive unless definite tubercles developed. This strict standard was necessary as an epidemic of pneumonia infection prevailed among our animals, causing pleurisy, pericarditis and peritonitis, as well as the pulmonary involvement which might simulate the more diffuse forms of tuberculosis. It was not possible with the assistance available to examine microscopically all the animals used, but tissues from doubtful cases were sectioned and examined for typical structural changes, and smears were stained and searched for tubercle bacilli. We attempted to use a sufficient number of dilutions so that at one extreme all results should be positive, while at the other they should all be negative. With some reagents that has been impossible.

Table 1 shows that phenol in 5 percent solutions is an efficient tuberculocide and nearly as efficient in 1 percent solution, though one of the guinea-pigs developed tuberculosis from bacilli which had been treated for one hour with 1 percent phenol in aqueous solution. It may be stated that guinea-pigs were also inoculated with bacilli which had been exposed only five minutes to phenol solutions with results practically the same as from a longer exposure. Formaldehyd kills human tubercle bacilli in 5 percent and 1 percent solutions, and shows considerable bactericidal power down to 0.05 percent solution. Ethyl alcohol kills tubercle bacilli in dilutions down to 25 percent, but below that it cannot be depended on. The tuberculocidal power of $CuCl_2$ is low, 10 percent and 5 percent in twenty-four hours killing the organisms when dried in a thin layer on the garnets; but even a practically saturated 25 percent solution fails to destroy the organisms in small clumps. $HgCl_2$ has a fairly high bactericidal power; 0.04 percent destroys the power of growth in the test-tube, but not the power of growth in the animal body. After twenty-four hours' exposure however, a dilution as high as 0.0001 percent destroys the power of growth even in the animal body. No higher dilutions have been tried, so that even weaker solutions might have the same effect. The $HgCl_2$ was on all cases neutralized with ammonium sulphid. One percent gold chlorid was strongly reduced, and deeply colored the bacterial clumps. The bacilli exposed for either one hour or twenty-four hours to 1 percent solution failed to grow either on glycerin agar or in the animal body. One-tenth percent also killed

TABLE 1

RESULTS OF EXPERIMENTS WITH DISINFECTANTS ON TUBE AND ANIMAL CULTURES

Disinfectant	Time	Dilutions																																					
		0.0001%		0.001%		0.002%		0.004%		0.005%		0.01%		0.02%		0.025%		0.04%		0.05%		0.075%		0.1%		0.3%		0.5%		0.8%		1%		5%		10%		25%		50%		75%		85%		95%		100%	
		T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
Phenol	1 hr.	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	+	++	+	++	+	−−	−	−−	−	∓∓	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	±	++	..	....	−	....	−	−−	−	−−	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	+	±±	−	−−	−	−−	−	−−	−	−−	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
Formol	1 hr.	..	....	..	++	..	....	..	....	..	....	+	++	..	....	..	....	..	....	+	±±	..	....	+	++	+	−−	−	∓∓	−	−−	−	−−	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	..	....	..	....	..	....	..	....	..	....	+	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	−	....	−	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	++	..	....	..	....	..	....	+	−−	..	....	..	....	..	....	−	−−	..	....	−	−−	−	∓∓	−	∓∓	−	∓∓	−	−−	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
Alcohol	1 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	....	+	++	−	−−	−	−−	−	−−	−	−−	−	....
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	....	±	....	−	....	−	....	−	....	−	....	−	....
	24 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	±	....	±	−−	−	−−	−	−−	−	−−	−	−−	−	....
Acetone	1 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
Chloroform	1 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
	24 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
Ether	1 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++
Toluene	1 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	±	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	++
Lugol's	1 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	±±
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	∓	−±
Cu Cl_2	1 hr.	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	+	++	..	....	+	++	+	++	+	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	..	....	..	....	..	....	..	....	+	....	..	....	..	....	..	....	..	....	+	....	..	....	..	....	..	....	+	....	..	....	+	....	+	....	+	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	+	++	..	....	..	....	..	....	+	±±	..	....	+	....	+	−−	+	....	..	....	..	....	..	....	..	....	..	....
Hg Cl_2	1 hr.	..	....	+	++	+	±±	+	∓∓	..	....	±	++	−	++	..	....	−	++	..	....	..	....	..	....	−	−−	..	....	..	−−	..	....	..	−−	..	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	..	....	+	....	+	−−	+	−−	..	....	−	++	−	−−	..	....	−	++	..	....	..	....	..	....	−	∓ ∓	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	..	....	+	−−	+	−−	−	−−	..	....	−	∓∓	−	−−	..	....	−	−−	..	....	..	....	..	....	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
Au Cl_3	1 hr.	+	++	+	++	..	....	..	....	+	++	+	±±	..	....	..	....	..	....	..	....	..	....	−	∓∓	..	....	..	....	..	....	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	+	++	+	++	..	....	..	....	+	−−	−	∓∓	..	....	..	....	..	....	..	....	..	....	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
Au $(CN)_3$	1 hr.	+	++	+	++	..	....	..	....	..	....	+	++	..	....	..	....	..	....	..	....	..	....	+	+∓	..	....	..	....	..	....	+	+∓	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	+	++	+	+±	..	....	..	....	+	+−	..	....	..	....	..	....	..	....	..	....	..	....	+	−−	..	....	..	....	..	....	+	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....
Ag NO_3	1 hr.	+	....	+	++	..	....	..	....	..	....	+	++	..	....	..	++	..	....	±	++	..	++	∓	++	..	....	−	....	..	....	−	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	6 hr.	+	....	+	....	..	....	..	....	..	....	+	++	..	....	..	++	..	....	−	++	..	....	−	++	..	....	−	....	..	....	−	....	..	....	..	....	..	....	..	....	..	....	..	....	..	....
	24 hr.	+	....	+	++	..	....	..	....	..	....	±	−−	..	....	..	−−	..	....	−	−−	..	++	−	....	..	....	−	....	..	....	−	−−	..	....	..	....	..	....	..	....	..	....	..	....	..	....

TABLE 3

COMPARISON OF THE RESULTS OF OTHERS ON THE DISINFECTANT ACTION OF THE SUBSTANCES USED IN THE EXPERIMENTS ON THE TUBERCLE BACILLUS

	Phenol		Formaldehyd		Alcohol		Chloroform		Ether		Acetone		Toluol		Lugol's		Cu Cl		Hg Cl		Au Cl		Au (CN)		Ag NO	
Organisms	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time	Percentage	Time
Streptococcus	5	1 min.	40	1 min.	5	20 hr.	100	1 min.	100	1 min.	....	....	10	48 hr.	1-1/400	1 min.	0.1	20 hr.	0.2	30 min.	....	....	....	....	....	....
			0.1	20 hr.	30	10 min.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Gonococcus	5	1 min.	40	1 min.	10	30 min.	100	1 min.	100	1 min.	....	....	0.25	15 min.	1-1/400	1 min.	0.1	20 hr.	0.2	30 min.	....	....	....	....	0.1	1 min.
	1	30 min.	0.1	20 hr.	20	10 min.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
M. pneumoniae	5	1 min.	40	1 min.	30	20 hr.	100	1 min.	100	1 min.	....	....	....	....	1-1/400	1 min.	0.1	20 hr.	0.2	30 min.	....	....	....	....	0.1	1 min.
			0.1	20 hr.	70	1 min.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
B. typhosus	.7-.8	15 min.	40	1 min.	30	10 min.	100	1 min.	100	1 min.	40	3 hr.	0.25	5 min.	1-1/400	1 min.	0.1	20 hr.	0.2	30 min.	....	....	....	....	0.1	1 min.
	5	1 min.	0.1	20 hr.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
B. coli	5	5 hr.	1	3 hr.	5.6	....	1.5†	....	4†	....	7.2†	....	10	4 hr.	....	....	....	....	1	3 hr.	....	....	....	....	0.1	1 min.
			4	1 hr.	....	....	0.6‡	....	2.3‡	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Staphylococcus	0.9	15 min.	....	....	....	....	0.6‡	....	...	....	....	....	10	17 days	....	....	3.36	‖	27	9 hr.	....	....	....	....	....	....
B. anthracis (spores)	5	2 hr.	5	1 hr.*	....	....	...	....	...	....	....	....	....	....	....	....	25		1.69	12 min.	3.4°	33 hr.	....	....	0.08	3 min.
																			0.42	30 min.	....	....	....	....	....	....
B. tuberculosis	1	5 min.	0.3	1 hr.	25	1 hr.	...	....	...	....	....	....	0.5	24 hr.	1-2/300	....	5	24 hr.	0.1	1 hr.	1	1 hr.	0.1	24 hr.	44.25	2 hr.
													100	....	§	....	....	....	0.001	24 hr.	0.005	24 hr.	....	....	0.01	24 hr.

* Seven colonies. † Solution. ‡ Vapor. § Not killed in 24 hours. ‖ Did not kill in 10 days. ° ($HAuCl_4$.)

the organisms dried on beads in twenty-four hours, but did not have that power over clumps of bacilli, nor in a shorter time. $Au(CN)_3$ in spite of its strong inhibiting power, as reported by Rosenthal, has even less bactericidal power than $AuCl_3$. One hundredth percent solutions of $AgNO_3$ kill tubercle bacilli in twenty-four hours, but the action of this salt is relatively slow, and twenty-four hours is required to prevent the growth in the animal body of even those bacilli exposed to 1 percent solutions. The clumps, which are deeply stained by the silver, will not readily grow on artificial media.

Table 2 represents the lowest molecular concentration at which the tubercle bacilli were killed. On a molar as well as on a per-

TABLE 2

LOWEST MOLECULAR POWER AT WHICH THE TUBERCLE BACILLI WERE KILLED

Molecular Concentration	Tube Cultures		Animals	
	1 Hour	24 Hour	1 Hour	24 Hour
0.00004	....	.....	.....	$HgCl_2$
0.00006	.....	.....	.....	$AgNO_3$
0.00015	.....	$HgCl_2$	.....	$AuCl_3$
0.0003	.....	$AuCl_3$	.....	.....
	.....	$AgNO_3$	.....	.....
0.0007	$HgCl_2$	.....	.....	.....
0.003	$AgNO_3$	.....	.....	Formaldehyd
0.004	.....	.....	.....	$Au(CN)_3$
0.015	.....	.....	$HgCl_2$	.....
0.02	.....	Formaldehyd	.....	.....
0.03	$AuCl_3$	Phenol	$AuCl_3$	Phenol
0.05	.....	.....	Phenol	Toluol
0.08	Phenol	.....	.....	.....
0.1	.....	.....	Formaldehyd	.....
0.17	Formaldehyd	.....	.....	.....
0.76	.....	.....	.....	$CuCl_2$
4.04	Alcohol	Alcohol	Alcohol	Alcohol

centage basis, as may be seen from the table, $HgCl_2$ holds the highest place as a tuberculocide. This salt has the highest disinfectant power of any of the metallic salts used by us. Koch,[15] in his work on disinfection in 1881, also assigns the highest disinfectant value to $HgCl_2$. Post and Nicoll[16] assign it a similar place in their experiments with rapidly growing organisms, but they do not neutralize nor even wash out their disinfectant and so inhibition must be added to the bactericidal effect. Krönig and Paul, however, using the garnet method and neutralizing the disinfectant, and then thoroughly washing the garnets, found that 0.42 percent $HgCl_2$ killed anthrax spores

15. Reviewed in *Mitt. a. d. Kaisl. Gsndthtsamte.*, 1881, p. 234.
16. *Jour. Am. Med. Assn.*, 1910, 55, p. 1635.

in thirty minutes, while it required two hours for 4.25 percent $AgNO_3$ to kill them, and 3.36 percent $CuSO_4$ did not kill them all even after ten days' exposure. Neither of these workers report results with the gold salts, and so no comparison can be made. Krönig and Paul, however, state that gold cyanid has very little disinfectant power and that gold chlorid has more influence. This statement corresponds with our findings regarding the tuberculocidal action of these gold salts.

Table 3 gives in brief the results which we have been able to find in the literature on the disinfectant action of the substances used by us, compared with our own results on the tubercle bacillus. In these reports of other workers, the methods vary greatly, and inhibitory and disinfectant influence are not always sharply distinguished. Leaving these points out of consideration, the bacillus tuberculosis appears less resistant to phenol, formaldehyd, $HgCl_2$, $AgNO_3$, and to $AuCl_3$ than the other organisms in the table, if we may compare its action to that which Krönig and Paul give for $HAuCl_4$, which is probably a dissociation product of $AuCl_3$. On the other hand, the tubercle bacillus is more resistant to alcohol, chloroform, ether, acetone, toluol, and the iodin-potassium-iodid mixture known as Lugol's solution. Omitting phenol of the first group and the iodin solution of the second, we may say that the human tubercle bacilli are less resistant to the disinfectants which are not fat-soluble, and more resistant to those which are especially fat soluble. The phenol exception may be explained by Cooper's statement[17] that the disinfectant action of phenol is due to its power to precipitate the proteins of the bacterial cell.

It has long been known that tubercle bacilli were especially rich in fats. Wells[18] states that different workers find an amount of substance soluble in fat solvents varying from 20 to 40 percent of the weight of the bacilli. Kresling[19] found the following percentages of chloroform soluble material:

Free fatty acid	14.38
Neutral fats and fatty acid esters	77.25
Alcohols from fatty acid esters	39.10
Lecithin	0.16

Deycke[20] states that these bacteria have not only a fatty sheath but also fat penetrating the body. He regards acid fastness as due to free fatty acids and finds that tubercle bacilli freed from their fats are no longer acid fast.

On the basis of their large fat content, it has naturally been assumed that fat solvents would more readily penetrate these organisms. It was shown by Sher-

17. *Jour. Biochem.*, 1909, 7, p. 175.
18. Chemical Pathology, 1914, Chap. 2.
19. *Centralbl. f. Bakteriol.*, Abt. 1, Orig., 1901, 30, p. 897.
20. *München. med. Wchnschr.*, 1910, 57, p. 633.

man[21] that fat soluble dyes, while readily staining masses of tubercle bacilli, almost entirely failed to stain individual organisms, and it was shown by De Witt[22] that many non-fat-soluble dyes, as the methylene blue group, penetrated and stained the individual tubercle bacilli very well. Benians[23] stated that tubercle bacilli were killed if shaken for one hour with 10 percent of toluene, while the bacillus coli required four hours, and the staphylococcus aureus seventeen days, with 10 percent toluene. Staphylococci were killed after being exposed for twelve days to benzol and five weeks to xylene, and the bacillus coli was killed after being shaken with benzol one hour, and five hours with xylene. Sata[24] and Ritchie[25] were unable to demonstrate fats in the staphylococcus aureus by the use of fat dyes, except when grown on glycerin agar, and Sata found no fat in the bacillus coli when grown on any media. Wells and Corper[8] also found that toluene water killed tubercle bacilli. These results but strengthened the idea which was expressed in Benians' paper that "bacteria having an available lipoid moiety are readily destroyed by toluene, and those not having such a moiety escape its action." On the other hand, Rosenau[26] states that it is doubtful whether the fatty substances of the tubercle bacillus protect it against external influences, and Bürgi[27] says that the connection of lipoid solubility with disinfectant power has not been proved.

Our own results with toluene have been negative with the exception of the 0.5 percent, which in twenty-four hours was able to destroy the organisms on the garnets so that they failed to infect guinea-pigs. As toluene is but slightly soluble in water this was a super-saturated solution, or rather an emulsion. The pure toluene in the same time failed to kill the tubercle bacilli even in a thin layer on the garnets. These results with the toluene emulsion in water agree, then, fairly well with those of Benians, and of Wells and Corper. It is difficult to understand why the pure toluene should not have had a similar effect, unless, as has been suggested, the pure toluene may have precipitated a layer on the surface and so it was unable to penetrate to the deeper layers. The tube cultures of the bacteria, exposed to full strength toluene showed but little development, and so considerable tuberculocidal action must be assigned to toluene. Chloroform, ether, and acetone, however, had no such contradictory action. The tube cultures of the organisms exposed to full strength acetone and chloroform showed only slight growth, but all animals developed local and general tuberculosis. Therefore the disinfectant action of these fat solvents must be considered as lower on tubercle bacilli than on fat-poor organisms, and much lower on tubercle bacilli

21. *Jour. Infect. Dis.*, 1913, 12, p. 249.
22. *Jour. Infect. Dis.*, 1913, 12, p. 68.
23. *Ztschr. f. Chemoth.*, 1913, Orig., 2, p. 40.
24. *Centralbl. f. allg. Pathol.*, 1900, 11, p. 97.
25. *Jour. Path. and Bakteriol.*, 1904, 10, p. 334.
26. *Bull. Hyg. Lab., U. S. P. H. and M. H. S.*, 1909, No. 57.
27. Handb. d. path., Mikroorganismen, 1913, p. 534.

than that of many reagents which are insoluble in fats. For this reason such disinfectant action as toluene has on tubercle bacilli must be ascribed to some other cause than solubility.

Considerable question has been raised in regard to the concentration of alcohol which has bactericidal influence, Beyer[28] stating that dilutions from 60 to 80 percent by weight have the greatest disinfectant value, and that below 60 percent and above 80 percent there is practically no disinfection. He found that silk threads infected with the staphylococcus aureus and exposed for six days to absolute alcohol, protected from evaporation, were uninjured. Schaumberg[29] suggested, as an explanation of the unfavorable results previously obtained with absolute alcohol, that alcohol precipitates the proteins on the surface and thus prevents penetration to the deeper layers. He finds that if the organisms are exposed in the form of an emulsion they are quickly killed by absolute alcohol, although, considering the form in which the organisms are exposed, the "absolute"-ness of the alcohol might well be questioned after mixture with the emulsion.

Krönig and Paul[14] concluded from a long and exhaustive series of experiments, that disinfectant action of many substances, especially acids, alkalies and metallic salts, depended on dissociation. This statement has been accepted by most of the disinfection workers since that time. Burgi[27] states that dissociation forms the most important preliminary in the disinfectant action of electrolytes. In easily dissociating mercury preparations, he says, the mercury ions disinfect most, the anions have the second place in influence, and the non-dissociated fraction of the molecule stands lowest in disinfecting power. There are, however, complicated organic mercury preparations in which the whole molecule has strong bactericidal influence. Reichel[30] refers the disinfectant action of $HgCl_2$ to the "Hg ion, whose strong affinity for proteins must lead to an irreversible reaction." Unfortunately, in the case of most of the metallic salts used by us, no reliable data are at hand regarding their degree of dissociation. Doubt has been cast on the accuracy of some of the earlier work, especially as regards the mercury salts. Chick gives the Hg ion concentrations, as obtained from the tables of Luther and Kahlenberg, as follows:

Percentage	Hg ions
0.1	63.0
0.05	57.5
0.01	42.5
0.005	37.0
0.001	23.0
0.0005	16.5

It is generally recognized that the degree of dissociation of $HgCl_2$ is by no means proportional to its concentration, and Chick and Martin[31] state that 100 percent increase in percentage concentration of the salt increases the Hg ion concentration only about 15 per cent. Later work has also shown that the electrolytic dissociation is not at all so simple as was earlier thought, and that many of the metallic salts, notably those of gold and of mercury, form many complex ions instead of, or as well as, the simple metal ion and the anion.

28. *Ztschr. f. Hyg. u. Infectionskrankh.*, 1911, 70, p. 225.
29. *Deutsch. med. Wchnschr.*, 1912, 38, p. 403.
30. *Biochem. Ztschr.*, 1909, 22, p. 149.
31. *Jour. Hyg.*, 1908, 8, p. 654.

Krönig and Paul as early as 1897 recognized that the gold salts dissociated but little, an observation which has recently been verified to the extent that probably but few simple gold ions are formed in the process of electrolytic dissociation. Data are given, which are as yet accepted, on the degree of dissociation of $AgNO_3$ and to some extent of $CuCl_2$.

	Percentage of Concentration	Molecular Concentration	Degree of Dissociation	Concentration of Metallic Ions
$AgNO_3$.....	1	.0058	95.28	.0056
	0.5	.0029	96.81	.0028
	0.1	.000588	98.72	.00057
	0.075	.000441	98.90	.00044
	0.05	.000294	99.10	.00029
	0.025	.000147	99.41	.00015
	0.01	.000059	99.71	.00059
$CuCl_2$.....	25.99	1.524	34.1	.5197
	12.995	.762	50.1	.3818
	2.6	.152	71.2	.1082
	1.3	.076	77.6	.0045
	.26	.015	90.1	.0135

These figures are too few to make comparisons safe and no statement could be made, based on our work as to whether the metallic ion is the true disinfecting factor or not. We may say only that many of the metallic salts, notably those of mercury and silver, are reliable tuberculocides, although they are slower than phenol, formaldehyd, and alcohol in their action.

SUMMARY AND CONCLUSIONS

Phenol in 5 per cent water solution kills human tubercle bacilli in five minutes, one hour, six hours, and twenty-four hours. It is nearly as efficient in 1 per cent solution, and shows some tuberculocidal action down to 0.1 per cent solution.

Formaldehyd in 1 per cent solution kills all tubercle bacilli in one hour (shorter time not tested). In 0.01 per cent solution it kills in twenty-four hours and so no disease develops in guinea-pigs. Formaldehyd, therefore, is somewhat more efficient than phenol.

Ethyl alcohol in 25 per cent solution kills all tubercle bacilli within one hour (shorter time not tried).

Acetone, chloroform and ether have very little, if any, tuberculocidal influence. Toluene and iodin show slight influence.

Of the metallic salts used, mercuric chlorid shows the greatest tuberculocidal action, 0.001 per cent killing in twenty-four hours, and 0.1 per cent. in one hour. Gold chlorid in 0.005 per cent solution

kills in twenty-four hours, while 0.025 per cent silver nitrate kills in the same time. One-tenth per cent gold tri-cyanid and 5 per cent copper chlorid kill the organisms in twenty-four hours.

From a comparison of the results of the experiments contained in this paper with those of disinfection work on other more rapidly growing organisms, the bacillus tuberculosis appears less resistant than the streptococcus, staphylococcus, pneumococcus or gonococcus, or than the bacillus typhosus, coli or anthracis spores, to phenol, formaldehyd, $HgCl_2$, $AgNO_3$, and $AuCl_3$, but more resistant than these other organisms to alcohol, chloroform, ether, acetone, toluene, and Lugol's solution.

The fat content of the tubercle bacillus does not determine its resistance to disinfectants. Our experiments seem to show that if the comparatively high content of this organism differentiates its behavior from that of bacteria of low fat content, it does so by rendering the tubercle bacillus more resistant to fat solvents, and less resistant to substances insoluble in fats.