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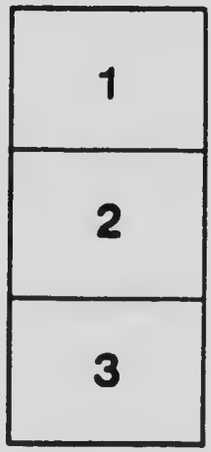


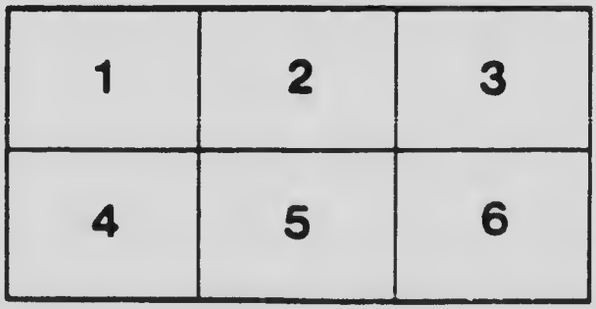

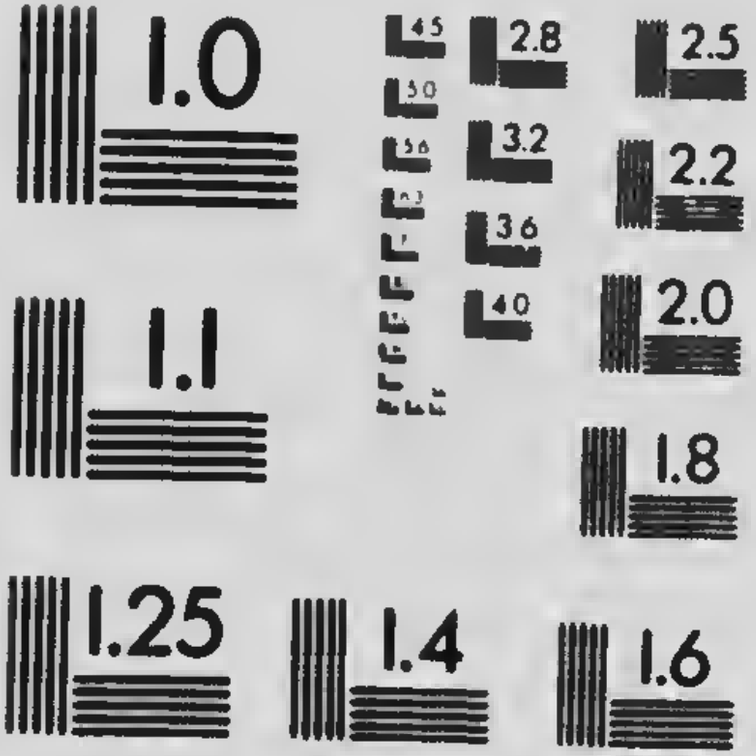


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PAPERS FROM THE CHEMICAL
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# No. 115: THE TOXICITY TOWARDS ANTHRAX AND STAPHYLOCOCCUS OF SOLUTIONS CONTAINING PHENOL AND SODIUM CHLORIDE, BY J. S. LEMON

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# THE TOXICITY TOWARDS ANTHRAX AND STAPHYLOCOCCUS, OF SOLUTIONS CONTAINING PHENOL AND SODIUM CHLORIDE

BY J. S. LEMON

The following experiments were carried out in the pathological laboratory of the University of Toronto in the winter of 1905–6, under the supervision of Prof. J. J. Mackenzie, with solutions supplied by Prof. W. Lash Miller.

Cultures of the "potato bacillus" (*B. mesentericus*) of anthrax (*B. anthracis*) and of *Staphylococcus pyogenes aureus* were grown on agar, the colonies washed off the agar, without breaking its surface, by 0.6 percent salt solution, and the suspension let stand (sometimes centrifuged) so that clumps might settle to the bottom. A second measured portion of the 0.6 percent salt solution was then infected by from one to three loops taken from the upper portion of the suspension; this constituted the "second suspension," in the case of potato spores and anthrax spores it was heated to 70° C to destroy vegetative forms. In the poisoning experiments, 10 cc of the toxic liquid was inoculated with a loopful of this "second suspension" and the time noted; to ensure that a good average sample should be removed for the inoculation, a mixing-rod was kept in the suspension tube, and the liquid was thoroughly stirred before a loopful was removed. The poison was then left to stand at room temperature, or in an incubator, and at measured intervals of time a loopful from it was added to 10 cc agar jelly, which had been melted and kept at 45° C; this was poured into a petri dish, allowed to solidify, and put away in the incubator. The number of colonies that grew on a measured area of the plate was taken as a measure of the number of cells left living in the poison at the time the agar was infected.

The usual precautions were taken against accidental infection tubes and instruments were sterilized, tubes plugged with cotton wool which was "flamed" before removing, etc.,

and the loopfuls of suspension were measured out with the "machine."[1] "Neutral agar" holding 1.5 percent agar-agar was employed, and the cultures were in all cases grown on the same agar as that used for the plates, for fear there might be some difference in the preparations made up at different times—although in every case the same recipe was followed. Koch has shown the need for this precaution in his experiments with iodine, which he found would act well in a neutral medium containing a trace of albumin, but not nearly so actively in an alkaline medium containing more albumin.

Preliminary experiments were, of course, necessary in order that the number of colonies grown on a plate might be suitable for counting. In the case of staphylococcus, for instance, one cc of 0.6 percent brine was added to a 24-hour-old pure culture and shaken; the second suspension was made by adding three loops of the first to 5 cc of the brine, and 10 cc agar was infected with one loop of this second suspension. The plates were so thickly sown that a count could not be made. The procedure was varied by using 5 cc salt solution for the first suspension and 10 cc for the second, but the plates were still too thickly sown. Finally, by using 10 cc for the first and 10 cc for the second, a satisfactory count of 7300 colonies was obtained; the plates had an area of 63 to 64 square centimeters, and the number of colonies on 12 cm of each plate was counted.

The spores were used instead of the vegetative forms of anthrax and potato bacillus because the former are much more resistant to phenol, and thus enabled the experiments to be made with higher concentrations of the poison. It was hoped that the innocuous potato spores might be used instead of the virulent anthrax, and preliminary experiments showed that insofar as their resistance is concerned, this is quite feasible; but it proved too difficult to prepare a uniform constant culture, while the very characteristic colonies of the anthrax made it easy to procure and maintain a pure strain, and in the end work with the potato bacillus was abandoned.

[1] Lash Miller: Jour. Phys. Chem., **24**, 563 (1920).

Spores of the potato bacillus were obtained from a pure culture on agar, by incubating at 34° C over night and then leaving four days at room temperature (19–20° C); anthrax spores by incubating the culture for 16 hours (when, according to Koch spores begin to form) and then letting stand at room temperature for 48 hours. This procedure worked well in practice, though some observers place the optimum for spore production (anthrax) at 31° C and others at 24–35° C.

### Resistance of the Spores to High Temperature

To compare their resistance to high temperatures, capillary tubes were filled with the second suspensions of potato spores, and anthrax spores respectively, their ends were sealed, and they were plunged into boiling water. After a measured interval they were removed, broken into sterile broth, and the growth noted. In the case of the potato spores there was a heavy growth after 2 minutes at 100° C, a smaller growth after 4 minutes, and a slight growth after 6 minutes, but none after 8, 10 or 15 minutes; in the case of anthrax, also, some were found alive after 6 minutes immersion in the boiling water.

### Effect of Temperature on the Rate of Poisoning by Phenol

That the resistance of the potato spore to phenol is much the same as that of anthrax, may be seen by comparing the following measurements with those given later for anthrax spores; the immediate object of this set, however, was to ascertain how much effect the temperature of the poison bath had on the time it took the potato spores to die. Solutions were made up containing 2.5, 3.0, and 3.5 percent of phenol, respectively, and 10 cc of each of them was inoculated with one loop of the potato spore suspension, and kept at room temperature for two weeks, plates being poured each day. As a control, one loop of the same suspension was added to 10 cc of 0.6 percent salt solution at the same time as the others. Counts of plates made immediately after inoculation showed no less numbers than the control. On the second day, a very slight reduction in the number of colonies was observed on

plates from the 3 percent and 3.5 percent phenol; on the third day the decrease was more marked, and there was also noticeable a decrease in the number of colonies on the plate from the 2.5 percent phenol. On the fourth day, all the plates still showed colonies, but many fewer than the control, especially in those infected from the 3 and 3.5 percent phenol. For seven days the numbers continued to fall off, but even after the poisons had acted for fourteen days, plating showed that some of the cells had survived.

The experiment was then repeated, using 0.6 percent salt, 3.5 percent phenol and 4.75 percent phenol, but keeping the poison tubes in the incubator at 34° C instead of at room temperature. The results were as follows (20 cm² counted on each plate):

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *B. mes.* in poison | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 23 | hours |
| 3.5% phenol | 121 | 108 | 58 | 57 | 46 | 38 | 15 | 9 | 0 | colonies |
| 4.75% phenol | 25 | 13 | 13 | 2 | 3 | 1 | 0 | 2 | 0 | colonies |
| 0.6% NaCl | 282 | — | — | 288 | — | — | — | — | 287 | colonies |

Thus a phenol solution that will not completely kill potato spores in fourteen days at 19 20° C will kill all but a few stragglers in eight hours at 34° C, and even these most resistant individuals in twenty-three hours.

With staphylococcus similar results were obtained (culture No. 6 used):

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Staph.* in poison | 0.5 | 10.5 | 20.5 | 31.5 | 45 | 55 | 60 | minutes |
| 0.6% phenol at 35.5° C | 30923 | 0 | 0 | 0 | 0 | 0 | 0 | colonies |
| 0.6% phenol at 24.5° C | 15064 | — | 3984 | 280 | 0 | 0 | 0 | colonies |
| 0.8% phenol at 35.5° C | | 0 | | 0 | 0 | 0 | 0 | colonies |
| 0.8% phenol at 24.5° C | | 112 | | 4 | 0 | 0 | 0 | colonies |
| 1.0% phenol at 35.5° C | 0 | 0 | 0 | — | — | | | colonies |
| 1.0% phenol at 24.5° C | 3 | 0 | 0 | | | | | colonies |
| 0.6% NaCl at 35.5° C | 23419 | | | | | | 22783 | colonies |

Another series with staphylococcus, culture No. 7, gave analogous results:

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Staph.* in poison | 2 | 5 | 7 | 10 | 20 | 30 | 35 | 40 | 50 | min. |
| 0.7% ph. at 34–35° C | — | 752 | 0 | 0 | 0 | 0 | 0 | — | — | col. |
| 0.7% ph. at 21–22° C | — | — | — | 21000 | 11264 | 4582 | 936 | 199 | 10 | col. |
| 0.8% ph. at 35° C | 17883 | 47 | 22 | — | 0 | — | — | — | 0 | col. |
| 0.8% ph. at 21–22° C | — | — | — | 14892 | 508 | 3 | 1 | 0 | 0 | col. |
| 1.0% ph. at 33–34.5° C | 1 | 0 | 0 | 0 | — | — | — | — | — | col. |
| 1.0% ph. at 21.5° C | — | — | — | 2 (12 min. 2 col., 15 m. 0 col.) | | | | | | |

## Effect of Salt on the Toxicity of Phenol towards Anthrax Spores

In the following tables the solutions are arranged in the order of the concentrations of the solutions of phenol in toluene with which they are in equilibrium; the numbers in brackets give the composition of the chemically equivalent solution of phenol, that is, the composition of the solution of phenol in water (without salt) which would be in equilibrium with the same toluene solution. For instance, after "1.9 percent ph. 5 percent NaCl" the number "(2.6)" signifies that a solution containing 2.6 grams of phenol, and no salt, per hundred cc, and a solution containing 1.9 g phenol and 5.0 g salt per hundred cc, would be in equilibrium with the same solution of phenol in toluene.

In the first set of experiments the poison tubes were inoculated from a suspension of anthrax spores that had been heated to 70° C for 30 minutes:

| *Anthr.* in poison | 18 m. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | hours |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.0% ph. + 5% NaCl (2.6) | 8 | 4 | 3 | 3 | 2 | 1 | 1 | 1 | 0 | colonies |
| 3.0% phenol | 6 | 3 | 2 | 1 | 3 | 1 | 1 | 0 | 0 | colonies |
| 3.5% phenol | 4 | 2 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | colonies |
| 2.5% ph. + 5% NaCl (3.6) | 4 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | colonies |
| 1.0% ph. + 10% NaCl (3.9) | 7 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | colonies |
| 4.5% phenol | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | colonies |
| 2.5% ph. + 10% NaCl (5) | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | colonies |

As it seemed probable that the low counts might be due to the prolonged heating at 70° C, in the next and in all subsequent work the suspensions were heated to 70° C for 20 minutes only. The counts so obtained were much higher; but that may be due in part at least to a change in the way of counting, for in the first series colonies growing at a depth in the agar had not been counted, they differed in appearance from those on the surface and it was feared that the culture was not pure.

| *B. anth.* in poison | 25 m. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | hours |
|---|---|---|---|---|---|---|---|---|---|
| 1.0% ph. + 5% NaCl (2.6) | 153 | 75 | 130 | 55 | 57 | 77 | 104 | 49 | colonies |
| 3.0% phenol | 182 | 174 | 101 | 88 | 97 | 89 | 75 | 56 | colonies |
| 3.5% phenol | 195 | — | 125 | 40 | 87 | 83 | 36 | 36 | colonies |
| 2.5% ph. + 5% NaCl (3.6) | 78 | 103 | 103 | 35 | 35 | 31 | 39 | 48 | colonies |
| 1.0% ph. + 10% NaCl (3.0) | 137 | 118 | 90 | 21 | 45 | 25 | 11 | 21 | colonies |
| 4.0% phenol | 103 | 107 | 118 | 44 | 30 | 50 | 35 | 25 | colonies |
| 2.5% ph. + 10% NaCl (5) | 72 | 40 | 3 | 5 | 1 | 0 | 1 | 0 | colonies |

In the third series the suspension was thoroughly centrifuged after heating:

| *B. anth.* in poison | 20 m. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | hours |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.0% ph. + 5% NaCl (2.6) | 142 | 134 | 114 | 84 | 46 | 18 | 13 | 23 | 6 | colonies |
| 3.0% phenol | 124 | 81 | 109 | 73 | 34 | 15 | 18 | 17 | 9 | colonies |
| 3.5% phenol | 119 | 107 | 90 | 48 | 12 | 17 | 10 | 5 | 0 | colonies |
| 2.5% ph. + 5% NaCl (3.6) | 145 | 154 | 90 | 112 | 15 | 15 | 14 | 10 | 3 | colonies |
| 1.0% ph. + 10% NaCl (3.0) | 115 | 92 | 41 | 51 | 7 | 5 | 7 | 2 | 0 | colonies |
| 4.0% phenol | 124 | 110 | 110 | 53 | 19 | 18 | 13 | 15 | 2 | colonies |
| 2.5% ph. + 10% NaCl (5) | 78 | 52 | 9 | 5 | 0 | 0 | 0 | 0 | 0 | colonies |

The fourth set were carried out under the same conditions as the third; the temperature of the incubator varied from 37.5° C to 42° C.

| *B. anth.* in poison | 15 m. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 hours | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.9% ph. + 5% NaCl (2.6) | 179 | 78 | 72 | 31 | 70 | 59 | 48 | 42 | 40 | colonies |
| 3.0% phenol | 118 | 184 | 149 | 79 | 61 | 52 | 75 | 75 | 55 | colonies |
| 3.5% phenol | 176 | 163 | 118 | 90 | 54 | 27 | 42 | 48 | 45 | colonies |
| 2.5% ph. + 5% NaCl (3.6) | 179 | 78 | 72 | 31 | 70 | 59 | 48 | 42 | 40 | colonies |
| 1.9% ph. + 10% NaCl (3.9) | 122 | 40 | 31 | 10 | 10 | 4 | 1 | 4 | 2 | colonies |
| 4.0% phenol | 169 | 115 | 58 | 64 | 10 | 24 | 16 | 17 | 11 | colonies |
| 2.5% ph. + 10% NaCl (5) | 90 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | colonies |

Comparison of these four sets, shows that even when working with every care, duplicate results are not to be expected; the trouble lies, no doubt, in the uneven distribution of the spores in the suspensions from which measured volumes are taken for inoculation and for culture. The results, however, leave no doubt that the toxicities of the phenol-salt solutions are about equal to those of their salt-free equivalents.

A fifth set was undertaken, including 1.22 percent phenol with 10, 15 and 20 percent salt. In this case the culture after 16 hours in the incubator was kept for 6 days at room temperature, the growth washed off as usual with 10 cc 2.6 percent brine, heated in the tube of a centrifuge for 30 minutes to 70° C, agitated to break up clumps, and then centrifuged at high speed. Two loops of this (first) suspension were used to inoculate 10 cc of each of the poisons, and after standing in a water bath (whose temperature varied from 38° C to 44° C) for the time noted, one loop was used to infect the 10 cc of agar; the "machine" was used, and the same small platinum tube that was used in the experiments with staphylococcus.

With the exception of the solution containing 1.22 percent phenol and 15 percent salt, the order of toxicity is that of the equivalent solutions; the results obtained with this exceptional solution are abnormal also in the relatively large count after one hour, followed by a rapid decrease to zero.

| *B. anth.* in poison | 1(?) | 2 | 3 | 4 | 5 | 6 | 7 | 8 hours |
|---|---|---|---|---|---|---|---|---|
| 1.22% ph. + 10% NaCl (2.1) | 1141 | 872 | 700 | 445 | 310 | 381 | 274 | 255 colonies |
| 2.5% phenol | 675 | 220 | 257 | 357 | 171 | 120 | 98 | 82 colonies |
| 3.0% phenol | 381 | 142 | 186 | 124 | 78 | 55 | 40 | 36 colonies |
| 1.22% ph. + 15% NaCl (3.0) | 117 | 40 | 10 | 2 | 3 | 0 | 0 | 0 colonies |
| 3.5% phenol | 140 | 106 | 41 | 25 | 25 | 16 | 13 | 11 colonies |
| 4.0% phenol | 58 | 14 | 34 | 32 | 16 | 8 | 8 | — colonies |
| 3.0% ph. + 5% NaCl (?) | 50 | 5 | 4 | 1 | 3 | 1 | 0 | 0 colonies |
| 1.22% ph. + 20% NaCl (7+) | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 colonies |

## Effect of Salt on the Toxicity of Phenol towards Staphylococcus

This form was chosen for the experiments with lower concentrations of phenol. Colonies on agar, 24 hours old, were washed off with 0.6 percent brine, and suspensions made as already described; of course, the heating to 70° C was omitted. All infections were made with the "machine," using a small platinum tube as "loop." The poison acted at room temperature; 0.6 percent NaCl was used as control; the various cultures are distinguished by numbers.

Preliminary experiments with 1.0 percent phenol showed that the time required to kill all the cells was the same whether the poison was infected by a large or a small number of cells, and that a culture 47 hours old gave about the same results as one 24 hours old.

| *Staph.* in poison | 5 | 10 | 15 | 20 | 25 | 30 | 35 | minutes |
|---|---|---|---|---|---|---|---|---|
| 1.0% ph., cult. No. 4, 24.5° C | 5 | 2 | 0 | 0 | 0 | 0 | 0 | colonie |
| 1.0% ph., cult. No. 5, 24.5° C | 8 | 2 | 0 | 0 | 0 | 1 | 0 | colonies |
| 1.0% ph., cult. No. 6, 24.5° C | 1402 | 29 | 0 | 0 | 0 | 0 | 1 | colonies |
| 1.0% ph., cult. No. 7, 24.5° C | 512 | 5 | 0 | 0 | 0 | 0 | 0 | colonies |
| 1.0% ph., cult. No. 9, 24.5° C | 2963 | 4 | 0 | 0 | 0 | 0 | 0 | colonie |
| 0.6% NaCl, cult. No. 7, 24.5° C | 20491 | 18073 | 16514 | | 15102 | | | colonie |
| 0.6% NaCl, cult. No. 9, 24.5° C | 15082 | 15048 | 13048 | | — | | | colonies |

With cultures Nos. 4 and 5, the machine was used; with the others the "biological lift," giving a much heavier infection. Culture No. 9 had been grown on agar for 47 hours; all the others in this paper for 24 hours only.

Experiments were then made to find the range of phenol concentrations within which poisoning takes place at a convenient rate; next the effect of sodium chloride, without phenol, was tested, and then comparison was made of the toxicity of 0.8, 0.7 and 0.6 percent phenol solutions with their chemical equivalents, viz., solutions containing 2.0 percent salt and 0.72, 0.63 and 0.54 percent phenol, respectively.

It soon became evident that experiments carried out under what were intended to be identical conditions, gave very different results. One cause of variation lay in the fact that equal volumes removed from the same suspension contain varying numbers of cocci - for instance, three plates prepared at the same time each from 10 cc of the same agar, infected in each case with the same volume of suspension (one loop taken with the machine), gave 5335, 5791, 6173 colonies, respectively, being a variation of 16 percent from the highest to the lowest. The principal cause, however, obviously lies in the variability of the staphylococcus itself, with culture No. 29 for instance, the time required for complete sterilization by 0.6 percent phenol was nearly twice as long as with culture No. 22, although in the first case the temperature was, if anything, a little higher.

In order to compare the toxicity of different solutions, therefore, it was necessary in every case to carry out simultaneous experiments with the same suspension; and as but little guidance could be obtained from previous experiments with the same poison but a different culture, a great many plates were poured which, on incubation, turned out to be sterile. All this added greatly to the amount of work and time required to obtain results; and if work of this kind is to be undertaken on any considerable scale, it will be necessary first to find some criterion of death which involves less delay than the plating method, and second to find conditions under which the

microbes experimented with can be grown "true to type" from the toxicological point of view. In comparison with the loss of time caused by the lack of these requisites, an uncertainty of 20 percent or so in the number of cells introduced into the poison is of little moment; and it is obvious that a more accurate regulation of temperature during the action of the poison can easily be attained by the use of a suitable thermostat.

SELECTION OF THE RANGE OF CONCENTRATIONS

| *Staph. No. 8* in poison | 0 | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 70 min. |
|---|---|---|---|---|---|---|---|---|---|
| 0.25% phenol, 24.5° C | — | — | 44077 | — | — | 55315 | — | 42995 | 59630 col. |
| 0.50% phenol, 24.5° C | — | — | 43084 | — | 51039 | 55032 | 49741 | 48129 | — col. |
| 1.0% phenol, 24.5° C | — | 16927 | 73 | 0 | — | — | — | — | — col. |
| 0.6% NaCl, 24.5° C | 39711 | — | — | — | — | 49724 | — | — | 43046 col. |

| *Staph. No. 10* in poison | 40 | 60 | 70 | 80 | 90 | 100 | 110 | 120 min. |
|---|---|---|---|---|---|---|---|---|
| 0.25% phenol, room temp. | — | — | 7000 | — | 7573 | — | 6427 | — col. |
| 0.5% phenol, room temp. | 10500 | 7500 | | 6637 | — | 6205 | — | 2418 col. |
| 0.6% NaCl, room temp. | 6045 | | | | — | 7064 | — | — col. |

| *Staph. No. 10* in poison | 130 | 140 | 150 | 160 | 170 min. |
|---|---|---|---|---|---|
| 0.25% phenol, room temp. | 5727 | — | 8591 | — | 7955 col. |
| 0.50% phenol, room temp. | | 1527 | — | 1209 | — col. |

In the experiments with culture No. 10, the room temperature varied from 20° C to 24° C, but as the tubes stood close together, and plates were poured from them alternately, the results are comparable.

**Comparison of 0.80% Phenol with Its Chemical Equivalent**

The equivalent contained 0.72 percent phenol and 2.0 percent salt. After "control" is given the number of colonies counted on plates from a 0.6 percent salt solution infected at the same time as the poison liquids; usually one plate from

this salt solution was poured immediately after inoculating, and another towards the close of the poison experiments.

Culture No. 17; temp. 22° C. Control: 7128, 8173 col.

| *Staph.* in poison | 5 | 6 | 10 | 11.5 | 15 | 16 | 20 | 21 | 30 | 40 | 50 | 67 min. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.80% phenol | | | | | | | | | | | | |
| | — | 8210 | — | 7191 | — | 6084 | — | 2864 | 734 | 44 | 0 | 0 col. |
| Equivalent | | | | | | | | | | | | |
| | 7058 | — | 5728 | — | 1819 | — | 2149 | — | 742 | 0 | 0 | 0 col. |

Culture No. 18; temp. 20° C. Control: 8546 after 3 min., 10882 after 5 min.

| *Staph.* in poison | 5 | 10 | 15 | 20 | 30 | 40 | 55 | 60 | 70 min. |
|---|---|---|---|---|---|---|---|---|---|
| 0.80% phenol | 10426 | 8400 | 6428 | 3755 | 350 | 6 | 0 | | col. |
| Equivalent | 10927 | 10692 | 6109 | 5543 | 2864 | 293 | 11 | 8 | 3 col. |

Culture No. 29; temp. 24 27° C. Control: 10500 col.

| *Staph.* in poison | 5 | 10 | 15 | 20 | 30 | 40 | 55 | 70 min. |
|---|---|---|---|---|---|---|---|---|
| 0.80% phenol | 3309 | 309 | 1 | 0 | 0 | 0 | 0 | 0 col. |
| Equivalent | 2546 | 55 | 38 | 4 | 0 | 0 | 0 | 0 col. |

Towards culture No. 17, the phenol and its equivalent proved equally toxic; but towards Nos 18 and 29, the "equivalent" was less toxic than the pure phenol solution.

### Comparison of 0.70% Phenol with Its Chemical Equivalent

The equivalent contained 0.63 percent phenol and 2.0 percent salt.

Culture No. 14; temp. 21° C. Control: 7828, 0555, 6491 col.

| *Staph.* in poison | 10.5 | 15 | 23 | 24.5 | 33 | 35 | 44 | 45 | (11 more, to 98) | min. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.70% phenol | | | | | | | | | | |
| | 3384 | — | 674 | — | 142 | | 3 | | 0 | col. |
| Equivalent | | | | | | | | | | |
| | 2262 | — | 970 | | 82 | — | 3 | | 0 | col. |

The following experiment was carried out at the same time, with the same culture and controls, to compare the effect of 0.6 and 0.7 percent phenol.

| *Staph.* in poison | 10 | 22.5 | 31 | 42 | 52.5 | 62.5 | 72.5 | 80 | 86.5 | 96.5 min. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% ph. | 5409 | 4975 | 3134 | 3039 | 2095 | 914 | 419 | 41 | 25 | 13 col. |

| Culture No. 14, *Staph.* in poison | 14 | 15 | 23-5 | 24 | 34-5 | 35 | 44 | 45 | etc., to 66 min. |
|---|---|---|---|---|---|---|---|---|---|
| 0.70% phenol | — | 1994 | 51 | — | 0 | — | 0 | — | 0 col. |
| Equivalent | 1980 | — | — | 311 | — | 9 | — | 0 | 0 col. |

Culture No. 16; temp. 22° C. Control: 5489 after 4 min., 5441 after 6 min.

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 85 | 100 min |
|---|---|---|---|---|---|---|---|---|---|
| 0.70% phenol | 2896 | 980 | 22 | 2 | 0 | 0 | 0 | 0 | 0 col. |
| Equivalent | 2604 | 2198 | 1604 | 1591 | 680 | 135 | 66 | 5 | 0 col. |

Culture No. 27; temp. 23-24° C. Control: avg. 9673 colonies

| *Staph.* in poison | 11 | 21 | 30 | 40 | 50 | 60 | 75 | 90 min. |
|---|---|---|---|---|---|---|---|---|
| 0.70% phenol | 1273 | 11 | 0 | 0 | 0 | 0 | 0 | 0 col. |
| Equivalent | 167 | 40 | 0 | 0 | 0 | 0 | 0 | 0 col. |

Culture No. 30; temp. 19-25° C. Control: avg. 10163 colonies

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 min. |
|---|---|---|---|---|---|---|---|---|---|
| 0.70% phenol | 7937 | 1400 | 37 | 0 | 0 | 0 | 0 | 0 | 0 col. |
| Equivalent | 1273 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 col. |

The five experiments with culture No. 14 were carried out at the same time and under the same conditions; those with Nos. 27 and 30, together with the experiments on 0.60 percent phenol given below. Towards cultures 14 and 27 the phenol solution and its equivalent are equally toxic; towards No. 16 the equivalent was very much less toxic, while towards No. 30 the equivalent seems to be somewhat more toxic than the pure phenol solution, though the unsteadiness of the temperature renders this conclusion uncertain. In comparison with the difference between the death rates with 0.7 percent and 0.6 percent phenol, however, the 0.7 percent phenol and its chemical equivalent come very close together, except in the experiments with culture No. 16.

### Comparison of 0.60% Phenol with Its Chemical Equivalent

The equivalent contained 0.54 percent phenol and 2 percent salt. Owing to the unexpected results obtained, a large number of experiments were made in the course of which fresh solutions were made up and the old ones were re-analyzed, the tubes, etc., used in the experiments were

exchanged for others, and every precaution taken to avoid accidental contamination; but the same general results were always obtained.

Culture No. 15; temp. 21° C. Control: 7471 after 2.5 min., 6627 after 5.5 min.

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 82 | 97 | 117 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 5844 | 5791 | 5955 | 3914 | 2810 | 2535 | 1889 | 1623 | 616 | 57 | col. |
| Equivalent | 7955 | 6351 | 7573 | 6151 | 5388 | 5476 | 5371 | 4484 | 1209 | 728 | col. |

Culture No. 19; temp. 23–27° C. Control: 3882, 5037 colonies

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 71.5 | 87.5 | 102.5 | min. |
|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 576 | 0 | 0 | — | 0 | 0 | 0 | 0 | col. |
| 0.60% equivalent | 3293 | 1591 | 1389 | — | 647 | 371 | 109 | 30 | col. |
| 0.70% equivalent | 1718 | 1256 | 627 | 84 | 1 | 1 | 0 | 0 | col. |

Culture No. 20; temp. 23.5. Control: 5728, 6713 colonies.

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75.5 | 91 | 105 | 121 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 4035 | 858 | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | col. |
| Equivalent | 6762 | 5473 | 3500 | 3946 | 3755 | 1909 | 1972 | 1095 | 389 | 170 | col. |

Culture No. 21; temp. 23.5° C. Control: 6619, 7636 colonies.

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | 120 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol (1) | 1284 | 585 | 74 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | col. |
| 0.60% phenol (2) | — | 763 | — | 1 | 0 | 0 | 0 | 0 | 0 | 0 | col. |
| Equivalent | 7319 | 5728 | 4811 | 4694 | 4137 | 3710 | 1654 | 1600 | 1336 | 332 | col. |

Culture No. 22; temp. 22–24° C. Control: avg. 6470 colonies

| *Staph.* in poison | 12 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | 120 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 5728 | 2482 | 338 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | col. |
| Equivalent | 7433 | 6364 | 5855 | 5454 | 5465 | 5091 | 4614 | 2800 | 2600 | 1146 | col. |

Culture No. 23; temp. 23–27° C. Control: avg. 6250 colonies

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | 120 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol (1) | 4391 | 1745 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | col. |
| 0.60% phenol (2) | 3882 | 1852 | 317 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | col. |
| Equivalent | 6619 | 5537 | 4968 | 4960 | 4028 | 3946 | — | 1728 | 1209 | 440 | col. |

Culture No. 24; temp. 22.5–25° C. Control: 9835, 10424 colonies

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | 120 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.65% phenol (1) | 5727 | 4646 | 1146 | 104 | 2 | 0 | 0 | 0 | 0 | 0 | col. |
| 0.60% phenol (2) | 7637 | — | 2819 | — | 40 | 1 | 0 | 0 | 0 | 0 | col. |
| 0.54% phenol | 5307 | 4072 | 2545 | 500 | 3 | 0 | 0 | 0 | 0 | 0 | col. |
| 0.60% equivalent | 8057 | 4455 | — | 1464 | 272 | 21 | 5 | 0 | 0 | 0 | col. |

Culture No. 25; temp. 20.5 24.5° C. Control: avg. 11137

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | 120 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 9164 | 7510 | 2927 | 305 | 1 | 0 | 0 | 0 | 0 | 0 | col. |
| 0.60% equivalent | 8782 | 8591 | 4136 | 955 | 83 | 1 | 0 | 0 | 0 | 0 | col. |
| 0.54% phenol | 8973 | 8591 | 7700 | 5918 | 580 | 109 | 5 | 0 | 0 | 0 | col. |

Culture No. 27; temp. 23 24° C. Control: avg. 9673 colonies

| *Staph.* in poison | 11 | 21 | 22 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | min. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 9037 | — | 7828 | 6619 | 1856 | 1044 | 425 | 1 | 0 | 0 | col. |
| Equivalent | 7700 | 5155 | — | 2757 | 1686 | 636 | 283 | 63 | 29 | 0 | col. |

Culture No. 30; temp. 19 25° C. Control: avg. 10163 colonies

| *Staph.* in poison | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 90 | 105 | min. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.60% phenol | 10100 | 7541 | 6746 | 2227 | 573 | 127 | 4 | 0 | 0 | col. |
| Equivalent | 10100 | 5300 | 2227 | 11 | 39 | 15 | 1 | 0 | 0 | col. |

Towards Culture No. 30, where the unsteadiness of the temperature renders conclusions uncertain, the equivalent solution was more toxic than the 0.60 percent phenol. In every other case, the equivalent proved less toxic often very much less toxic than the pure phenol solution. In the case of No. 19, the solution containing 0.60 percent phenol was even more toxic than a solution containing more phenol and salt as well, viz., "the 0.70 percent equivalent" 0.61 percent

phenol + 2.0 percent salt; similarly with No. 24, a solution containing 0.54 percent phenol proved more toxic than one containing 0.54 percent phenol + 2.0 percent salt, but with No. 25, the reverse was the case. Towards culture No. 25, the 0.60 percent phenol was distinctly more toxic than the 0.54 percent phenol solution, while towards No. 24 there was no great difference, in the behaviour of the two solutions.

## Summary

My experiments with anthrax spores show that the increased toxicity observed on adding salt to a phenol solution is in accordance with the assumption that two solutions of phenol, with or without salt, are equally toxic if their compositions are such that both would be in equilibrium with the same solution of phenol in toluene. The experiments with staphylococcus, however, in which lower concentration phenol were employed, show that while the asumption fairly in accord with the behaviour of 0.80 percent phenol in the case of 0.60 percent phenol the chemically equivalent solution containing salt is much less toxic; 0.70 percent phenol occupies an intermediate position.

Every care has been taken to avoid accidental contamination of the vessels, and accidents in making up the solutions; and in view of the large number of corroborative experiments, the general result must be regarded as well established; but I have had no time to look farther into its cause.

*The University of Toronto*
*June, 1920*

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