A COMPARISON BETWEEN THE GERMICIDAL POWER OF A DISINFECTANT IN SOLUTION AND IN THE EMULSIFIED STATE.

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(1 Text Figure.)

In the Milroy Lectures of this year, Professor Hewlett stated[1]:—"There is some evidence to show that the germicidal power of emulsified disinfectants is greater than can be accounted for by the activity of the active constituent and theoretically seems likely, but we want some reliable experimental work in confirmation. It is true that Chick and Martin[2] have compared the germicidal value of higher tar acids emulsified in water and dissolved in alcohol, their results showing that the emulsified form was much more active; but the reliability of this work is vitiated by the fact that they have entirely failed to take into account the influence of alcohol in decreasing germicidal activity; for example in the case of phenol, which I dealt with in my last lecture."

Rideal and Walker[3] also claim that an emulsion of trikresol has a germicidal power three times greater than a solution of the same concentration, but in this case the comparison was imperfect, a resin soap being used for the emulsion and an oleate for the solution.

The factors determining the accuracy of disinfectant experiments are so delicate that concordant results can only be obtained when the conditions under which the comparison is instituted are precisely similar.

It is obviously impossible to prevent a soluble substance from going into solution; that is, without changing its composition, and thus altering its properties; the most that can be hoped for, is that the rate of solution will be retarded. The range of solvents, also, is limited, since

[1] *Lancet*, 1909, I. p. 893.

[2] *Journ. of Hygiene*, 1908, VIII. p. 698.

[3] *Journ. Sanitary Inst.*, 1903, XXIV. p. 425.

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many have, in themselves, antiseptic or germicidal action; and, as the substance will only remain in the state of suspension for a short space of time, it is necessary that the diluent should be inert for the difference to become appreciable.

A weak emulsion of tragacanth, when kept between 20° and 40° C. was found to delay solution of phenol for about 15 minutes, and, in the case of the difficultly soluble cresols, the particles could be detected after 3 hours.

It also has the merit of being perfectly homogeneous, and thus does not depreciate the germicidal power, as particulate matter has been found to do. It is quite inert, and, by using the same sample of tragacanth, an emulsion, similar in properties, can be prepared for each experiment.

Accordingly, ·4 gm. of tragacanth (sterilised) was placed in a sterile graduated cylinder, and moistened with 2 c.c. of absolute alcohol; about 80 c.c. sterile water were now added, and the cylinder well shaken. The mucilage was then sterilised in an autoclave at 120° C. for 10 minutes and allowed to stand over-night, that the liquid might be freed from air bubbles. The volume was finally made up to 100 c.c. less the amount of disinfectant to be added.

This represented the basis for the emulsion. When preparing the solution, the disinfectant was dissolved in the sterile water before being added to the tragacanth, and, after standing some time, the product made up to 100 c.c. This was not sterilised in the autoclave.

After placing 20 c.c. of the tragacanth basis in a sterile Erlenmeyer flask, and withdrawing an amount equal in volume to that of the disinfectant to be used, the measured amount of cresol or phenol was added, and the whole gently rotated. A known quantity of the organism was quickly added and subcultures were made in the Rideal-Walker manner, strict precautions being taken to ensure constancy in time, in quantity added to each subculture (3 mm. loop bent at a right angle to the surface of the liquid), and in temperature.

To 20 c.c. of the solution the same amount of culture was added, and subcultures were made in a manner precisely similar to that used for the emulsion.

The following results were obtained:

Exp. I. Temp. 20° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask, and subcultured every 2 minutes.

A. 20 c.c. 1 % emulsion of phenol.

B. 20 c.c. 1 % aqueous solution of phenol (without tragacanth).

Result:—No growth in either case after 2 minutes.

EXP. II. Temp. 15·5° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask, and subcultured every 2 minutes.

A. 20 c.c. 1 % emulsion of phenol kills in 4 minutes.

B. 20 c.c. 1 % solution of phenol kills in 6 minutes.

EXP. III. Temp. 17° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask and subcultured every 60 seconds.

A. 1 in 120 emulsion of phenol kills in 6 minutes.

B. 1 in 120 solution ,, ,, 11 ,,

EXP. IV. Temp. 20° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask and subcultured every 30 seconds.

A. 1 in 120 emulsion of phenol kills in 4·5 minutes.

B. 1 in 120 solution ,, ,, 6 ,,

EXP. V. Temp. 18° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask and subcultured every 30 seconds.

A. 0·9 % emulsion of phenol kills in 1 minute.

B. 1 % solution ,, ,, 1 ,,

EXP. VI. Temp. 16° C.

A. 0·9 % emulsion of phenol kills in 1½ minutes.

B. 1 % solution ,, ,, 1½ ,,

It will be seen from the above that although the temperature and strength of disinfectant were varied, the emulsion was slightly stronger in each case. In order to demonstrate more clearly the difference in strength, a hardier organism (*B. coli*) was subjected to experiment.

EXP. VII. Temp. 20° C. 1 c.c. *B. coli* broth 24 hrs. old added to each flask, and subcultured every 30 seconds.

A. 1 % emulsion of phenol kills in 2½ minutes.

B. 1 % solution ,, ,, 4 ,,

It has been stated elsewhere that the phenol could be maintained in the emulsified state for a short time only. In order to lessen this difficulty, the more slowly soluble trikresol was used in the remaining experiments.

EXP. VIII. Temp. 18° C. 1 c.c. *B. coli* broth, 24 hrs. old, added to each flask and subcultured every 30 seconds.

A. 0·4 % emulsion of trikresol kills in 3½ minutes.

B. 4 % solution ,, ,, ,, 6½ ,,

A still more resistant organism is the *Staphylococcus pyogenes aureus*. A 24 hr. culture on slope agar was taken and emulsified with sterile water.

EXP. IX. Temp. 20° C. 1 c.c. suspension of Staphylococcus added to each flask, and subcultured on to slope agar at intervals of 30 seconds for 5 minutes.

A. ·5 % emulsion of trikresol.

B. ·5 % solution ,,

Result:—The death point was not reached, but the difference in the amount of growth was very marked, for whereas, that exposed to the action of the solution was vigorous, the colonies after treatment with the emulsion were small and scanty in number.

The uniform success attending these experiments suggested that a quantitative result might be obtained. It was recognised that although

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the emulsion would not persist until the end of the experiment, yet the ratio obtained during the first half would be maintained throughout. Accordingly a thin emulsion was made of an old sporing culture of *B. mycoides*, and a loopful of this added to the emulsion and solution of the disinfectant. Loopfuls were sowed into agar plates at intervals, and after incubation the colonies were counted.

In the first experiment, the emulsion was too thick and the plates so crowded that the individual colonies could not readily be enumerated. An appreciable difference was noted, and a rough count taken. On dividing the number of colonies from the solution by the number from the emulsion, an interesting ratio was obtained.

Time	Ratio $\frac{\text{Solution}}{\text{Emulsion}}$
After 1 hour	1·15
2 hours	2·24
3 ,,	1·68
3½ ,,	1·21
3¾ ,,	1·20
4 ,,	1·18

Temp. 40° C.

The steepness of the curve obtained from the results of the next experiment during the first two hours of action verifies these figures, and seems to indicate that the germicidal power gradually decreased as the trikresol passed into solution, which happened rather quickly in this experiment owing to the high temperature. The suspension of spores was therefore diluted and the experiment repeated, with the following results.

Spores of B. mycoides.

Temp. 30° C. 1 loopful of suspension added to each flask.

Time	Emulsion 2 % Trikresol No. of colonies	Solution 2 % Trikresol No. of colonies
1 hour	85	98
2 hours	52	72
2½ ,,	43	66
3 ,,	36	56
3¼ ,,	32	54
3¾ ,,	28	48
4½ ,,	21	37
5¼ ,,	15	31

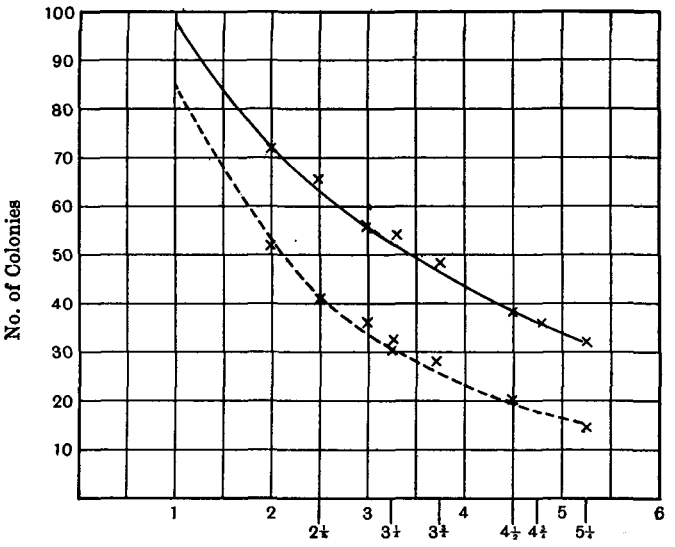
1 loopful 3 mm. diameter sowed into each plate. A graphic representation of these figures renders the difference more marked.

The course of action of a disinfectant on bacterial spores has been shown by Miss Chick[1] to progress according to the formula

$$K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2},$$

where n_1 and n_2 are the number of organisms surviving after times t_1 and t_2 respectively.

Disinfection of Spores of B. mycoides by an emulsion and by a solution of Trikresol. Temp. 30° C.



Time of exposure to action of disinfectant

—— curve of solution.
----- curve of emulsion.

When this is applied to the number of colonies given above the following constants are obtained.

K for emulsion	K for solution	Ratio $\frac{K \text{ from emulsion}}{K \text{ from solution}}$
·213	·134	1·59
·197	·114	1·72
·187	·121	1·54
·188	·115	1·6
·175	·113	1·54
·174	·121	1·44
·177	·117	1·51

[1] *Journ. of Hygiene*, 1908, VIII. p. 94.

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It will be noted that throughout the experiment the constant of the emulsion is higher than that of the solution, but the ratio of the two is nearly the same. Moreover there is a continued decrease in value as the process of disinfection goes on.

The experiments were identical in every respect, and done simultaneously; the larger value of K therefore indicates that the rate of disinfection is greater in the case of the emulsion. The even ratio may be accounted for by the fact that the actual germicidal action is similar in each case, and also that emulsion was not changing to solution.

The gradual decrease exhibited in both emulsion and solution must be the result of the number of spores being lessened; the survivors may be the more resistant forms.

No exact accuracy is claimed for the above figures, the purpose in view being simply to demonstrate the superior germicidal power of the emulsified trikresol, and not to indicate the exact rate of disinfection. The unequal distribution throughout the basis probably accounts for the lack of that symmetry which characterises the curves depicted by Miss Chick.

When a few drops of the emulsified disinfectant were examined under the microscope with a small quantity of typhoid culture, it was evident that the motility of the bacteria was diminished by the viscosity of the liquid. Clumping of the bacteria around a globule of disinfectant (trikresol) could be seen, and it is presumably owing to the fact that a greater concentration exists around these clumps than throughout the solution that the emulsified form exhibits an accelerated rate of action.

In conclusion, the writer's best thanks are due to Professor Hewlett, for continued help during the course of these experiments.