# A COMPARISON OF THE POWER OF A GERMICIDE EMULSIFIED OR DISSOLVED, WITH AN INTERPRETATION OF THE SUPERIORITY OF THE EMULSIFIED FORM.

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Henle (1889) found that "Creolin," an emulsion of tar acids, was a more efficient disinfectant than could be explained from observations upon each of its constituents separately, and concluded that the extra efficiency was connected with the emulsified form. Henle's conclusions do not appear to us to be necessarily justified by his observations.

Rideal and Walker (1903, p. 425) state that an emulsion of Trikresol is equal in germicidal power to a solution three times as concentrated, but give no details.

We have compared the germicidal value of higher tar acids when emulsified in water and when dissolved in alcohol. The spores of *B. subtilis* were chosen, because alcohol alone was found to have no action upon them within the time limits employed.

A suspension of an old sporing culture of *B. subtilis* was made in water and heated for some minutes to 80° C. By a preliminary plating experiment an amount of spores suitable for enumeration experiments was determined, and this amount was added to two tubes containing 2·5 % tar acids in solution in alcohol and emulsified in water respectively. The tubes were placed in a water bath at 20° C. and a definite quantity removed from each at known intervals of time, and plated. After 24 hours' incubation the numbers of colonies on the plates were counted.

The numbers of colonies formed a regularly decreasing series in both cases, but diminished more rapidly in the case of disinfection by tar acids in the form of an emulsion (see Table I).

Disinfection has been shown by Madsen and Nyman (1907) and by one of us (H. C. 1908) to proceed according to the equation

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

where $t_1$ and $t_2$ are any two times, and $n_1$ and $n_2$ the numbers of surviving organisms in unit volume of the liquid at times $t_1$ and $t_2$ respectively, and $K$ a constant depending on the particular organism and disinfectant, the concentration of the latter, and the temperature.

This constant is therefore an expression for the velocity of the reaction.

From the number of bacteria surviving after various intervals of time (see Table I, col. 4) two values of $K$ were calculated (see col. 5), the ratio of which expresses the relative velocity of the disinfection by the same concentration of tar acids in the two conditions.

The mean value for $K$ was 0·049 for the emulsion and 0·0066 for the solution, so that coal tar acids in the above concentration disinfected

TABLE I.

*Spores of B. subtilis.* 20° C.

| Disinfectant | Time elapsing, minutes | Amount of sample | Mean no. of bacteria present in 1 drop disinfecting mixture | $K$, assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$ $\left(K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}\right)$ |
|---|---|---|---|---|
| Exp. 29. 2. 08 | | | | |
| $2\frac{1}{2}$ % tar acids emulsified in water | 1 | 1 drop | 2750 | taken as an initial value of $n$ $(=n_1)$ in calculating values of $K$. |
| | 4·5 | 1 ,, | 1804 | 0·052 |
| | 20 | 1 ,, | 373 | 0·046 |
| | | | Mean value of $K$ | 0·049 |
| $2\frac{1}{2}$ % tar acids dissolved in alcohol | 1 | 1 ,, | 1750 | taken as an initial value of $n$ $(=n_1)$ in calculating values of $K$. |
| | 70 | 1 ,, | 607 | 0·0067 |
| | 121 | 1 ,, | 393 | 0·0054 |
| | 230 | 1 ,, | 32 | 0·0077 |
| | | | Mean value of $K$ | 0·0066 |

7·5 times[1] as quickly in the form of an emulsion as when dissolved in alcohol.

The explanation of this increased efficiency of a germicide in the emulsified form demands some consideration. Fowler (1907) suggested that there is a mutual attraction between the particles of an emulsion and the bodies of bacteria. He was led to this conclusion from the examination of stained microscopical preparations containing mixtures of bacteria and emulsions; he then observed bacteria with particles of disinfectant adhering to them. On repeating Fowler's observations it was found that methylene blue, in addition to staining the particles, de-emulsified the disinfectant, so that conclusions drawn from appearances seen after its addition are not free from fallacy.

In order to arrive at an understanding of what takes place, we observed mixtures of living bacteria (*B. paratyphosus*) and an emulsified disinfectant, under a magnification of 1100 diameters, using a dark background and side illumination. Under these circumstances the bacteria and the particles of the disinfectant appear as shining bodies and their relation to one another can be easily observed.

The particles of the emulsion, and to a lesser degree the bacteria, exhibited active Brownian movement. The bacteria, which were considerably larger than the mean diameter of the emulsified particles, were seen to be continually bombarded by the latter. The bacteria were thus frequently brought into intimate contact with particles of tar acid somewhat smaller than themselves, but whether any portion was left remaining upon them could not be directly observed. Nothing of the nature of an agglutination of the particles upon the bacteria was seen, although an occasional temporary attachment was noticed.

Nevertheless, whereas the emulsified disinfectant alone showed a multitude of small shining bodies, an emulsion of equal concentration to which bacteria had been added showed a great reduction in the number of these bodies, so that many of the emulsified particles of the disinfectant had been appropriated by the bacteria.

This appropriation by bacteria of particles of tar acids from an emulsion was readily demonstrated by mixing together a suspension of bacteria and an emulsion of tar acids, allowing them to remain in contact a short time, and subsequently centrifuging. Under these circumstances the mixture was completely cleared of all opacity when

[1] This does not mean that the disinfectant in the emulsified form was, weight for weight, 7·5 times as efficient, see "An Investigation of the Laws of Disinfection," by H. Chick, this *Journal*, pp. 117—132.

the relative quantities of tar acids and bacteria were suitably arranged. A control tube containing the emulsion showed very little settlement on centrifuging.

The suspension of bacteria (*B. paratyphosus*) employed contained 0·025 grm. dry weight of bacteria per c.c. When 1 c.c. of this bacterial suspension was mixed with 1 c.c. of an emulsion containing 0·6 in 1000 of tar acids, so that the 2 c.c. of mixture possessed a concentration of 0·3 per 1000, a perfectly clear solution was obtained after centrifuging, i.e. 0·025 grm. bacteria united with 0·0006 grm. of tar acids, which is more than 2 °/₀ of its own weight.

In order to gain some further insight into the nature of the process a series of quantitative experiments were undertaken using a constant weight of bacteria and emulsions of varying concentration. *Staphylococcus pyogenes aureus* was employed. The cocci were three times well washed with water and separated by means of a centrifuge. The final bacterial suspension contained 0·0785 grm. dry weight of cocci in 1 c.c.; 2 c.c. of this suspension were added to a series of tubes each containing 6 c.c. of an emulsion of tar acids, ranging in strength from 0·15 to 0·89 °/₀. The contents were well mixed and, after two hours, centrifuged. A measured quantity of the supernatant liquid was removed and acidified, the tar acids extracted with ether, and determined (see this *Journ.* p. 683).

The results are set out in Table II, and show that the emulsified tar acids removed by one gramme of staphylococci is at first roughly proportional to the original concentration of tar acids, but, as this concentration increases, its influence becomes progressively less and the amount removed rapidly tends to a maximum.

TABLE II.

*Adsorption of emulsified tar acids by bacteria.*

| | Organism employed | Concentration of bacteria dry weight °/₀ | Initial concentration of tar-acids °/₀ | Final concentration of tar-acids °/₀ | Amount adsorbed by 1 grm. dry weight bacteria, grms. |
|---|---|---|---|---|---|
| Exp. 19. 9. '08 | Morgan's | 1 | 0·714 | 0·414 | 0·300 |
| | Bacillus | 1 | 0·178 | 0·094 | 0·084 |
| Exp. 28. 9. '08 | *Staphylococcus* | 1 | 0·892 | 0·496 | 0·396 |
| | *pyogenes aureus* | 1 | 0·594 | 0·228 | 0·366 |
| | | 1 | 0·446 | 0·164 | 0·282 |
| | | 1 | 0·298 | 0·086 | 0·212 |
| | | 1 | 0·148 | 0·072 | 0·076 |

The results with bacteria are indeed similar to those obtained by us with animal charcoal (Table XI, this *Journ.* p. 684), but one gramme dry weight of bacteria removes, under similar conditions, about twice as much of the emulsion as was removed by the preparation of animal charcoal employed, viz. 39·6 % of its own weight.

We found that the cocci lost about 50 % of water on drying at 110° C. so that weight for weight bacteria and animal charcoal happen to be about equally effective. This similarity between the two processes is strikingly demonstrated if the figures for the amounts removed by one gramme of bacteria and charcoal respectively are both plotted against the original concentration of the emulsion. This has been done in Fig. 1, where the circles represent the amounts of tar acids adsorbed by one gramme charcoal and the crosses that removed by one gramme of bacteria in their natural condition. It will be seen that the curve drawn for the adsorption by charcoal would do almost equally well for that by the cocci.

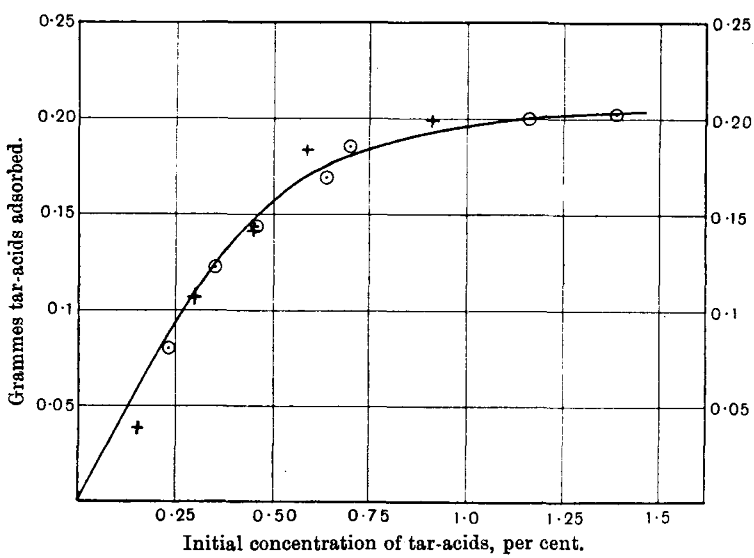


Fig. 1.

From these results there is little doubt that the removal of an emulsion of tar acids by bacteria is, in the first instance, a process of adsorption and not a chemical combination, and that disinfectants of this class possess superior efficiency, because owing to this adsorption

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the bacteria rapidly become surrounded by the disinfectant in much greater concentration than exists throughout the liquid. As has been pointed out elsewhere, however (this *Journ.* p. 689), the same property of adsorbing the particles of the emulsion is possessed by most organic particles, in consequence of which the germicidal value of this class of disinfectant is greatly deteriorated by the presence of particulate organic matter other than the bacteria which it is desired to destroy.

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