

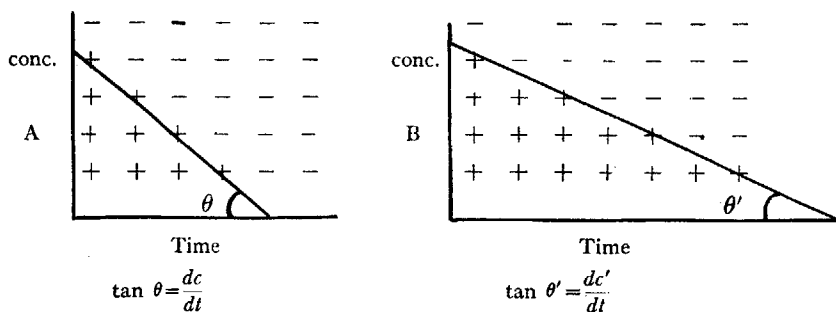
SOME REMARKS ON THE RIDEAL-WALKER TEST AND ON THE RIDEAL-WALKER METHOD. WITH SPECIAL REFERENCE TO THE "LIFE FACTOR" AND TO THE "MECHANICS OF DISINFECTION" AND THEIR INFLUENCE ON VELOCITY AND EQUILIBRIUM VALUES.*

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Within the last year or so several attempts have been made to measure the rate of disinfection, and incidentally several attacks have been made on the Rideal-Walker test. These can have arisen only through an insufficient appreciation of the Rideal-Walker method and what is claimed for it.

The Rideal-Walker test is a measure of the total possible germicidal work a disinfectant can do in terms of an arbitrary standard whose absolute value is unknown. This work is done under fixed conditions of temperature and time on a fixed working substance. (Whether this fixed working substance is homogeneous or not will be discussed later.)

No account, however, is taken of the velocity, although velocity measurements are easily obtained from the slope of the curves obtained in the Rideal-Walker method. Thus with the two disinfectants A and B in the diagrams, the velocities are:



This problem of rate the Rideal-Walker test apart from the method does not attempt to deal with for the following reasons:

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First, it is only in very rare cases that the time of partial sterilization is required, complete disinfection being generally desired, and second, although the integral death time is measurable, the fractional time or the rate of death is special for each definite set of circumstances and indeed for each experiment, as we shall endeavor to point out later. Those authors who have criticized the Rideal-Walker test from theories they have developed about rate measurements have attacked a method which is not intended to measure rates.

It is obvious that in order to determine a disinfectant velocity we have to measure (1) the number of bacteria left alive after a certain time, and (2) the number killed (which of course is easily ascertained if we know the number we started with). Madson and Nyman, Chick, Earle Phelps, and others in their valuable work on the measurement of the rate of death of bacteria have not yet come to a final conclusion. Miss Chick suggested that disinfection was similar to a unimolecular reaction and that it takes place in one direction only, namely, live organism + active disinfectant \rightarrow dead organism + active and inactive disinfectant. The velocity of the reaction would then be represented by the equation $\frac{db}{dt} = kb$, where b is the number of organisms present at any time in unit volume, and k the velocity constant.

Values of k which should be constant, depending only on the kind and concentration of the disinfectant and on the temperature and bacterial species, do not give concordant results. One must therefore attack the problem once more and find whether this equation meets the case and whether this is really the actual mechanism of the reaction. We have in this equation three factors to consider: the organism, the disinfectant, and the time. The time is known and controllable; we may therefore regard this factor as determined. The second factor is the organism. Microorganisms as a whole are susceptible to change. This is recognized in the Rideal-Walker test, which cultivates a known organism for a known time on a definite medium at a given temperature. This culture produces what might be called a community of organisms whose age, capabilities for work, etc., are just as variable and heterogeneous

as in a community of human organisms, but gives on the whole a fair average. Earle B. Phelps wishes to make a reduction of 50 to 75 per cent on the total number present as a standard. This is obviously wrong, as the "sick" or "starved" bacteria will die first and leave the hardy and virile ones which might need stronger and more prolonged doses of the disinfectant to kill them. This equality of the organism is the chief corner-stone of the unimolecular law hypothesis. If the bacteria all budded or spored at the same time, if the medium round them was perfectly homogeneous, if their parents were equally strong, if there was no heredity, if they never kept food or light from their neighbors, if the disinfectant were dealt out in equal measure to each microbe, if the osmotic pressure of each personage was the same, if each body was of equal area, if there were no deformed, if in fact microbes were ions or atoms, then the equation $\frac{db}{dt} = kb$ might perhaps hold true. In fact, as disinfection varies with the concentration the unimolecular formula is not applicable, and, as shown by Earle Phelps, k in the above formula is not a constant and has to be replaced by the more complicated expression KC^n where n is an exponent representing the order of the reaction.

To show what is meant by the changeability of a set of organisms, one might mention the change in the toxic value of diphtheria when grown in guinea-pigs or on agar, or the change in cholera vibrios if kept in water, or the resistance of anthrax after 10, 20, and 30 hours' culture. If an entire community can change, how much more can variations be found inside the community and especially in those extremely variable factors, namely, those of life and death.

While still dealing with the same point, it may be noticed that dead bacteria are not necessarily removed from the sphere of action. It is not to be supposed that the action of killing and making alive is a reversible one, but it has not been proved that dead bacteria are not still affected by the germicide. Rather, on the contrary, it has been shown that at least in a few cases sterile organic matter present lowers the Rideal-Walker figures, and certainly dead bacteria come within the scope of the term sterile organic matter.

The unimolecular equation assumes that no such disturbing factor should be present and this can be true only when the disinfectant is in such large excess that the addition of even very small traces of organic matter can make no difference, and this is not experimentally possible.

We have now to deal with the third and last factor, the disinfectant. It has been assumed, up to the present, that all disinfectants act in the same way. There is no foundation for this belief.

The first essential of a disinfectant is that it must come in contact with the organism. We must assume that this takes place and discuss the next proceeding from this premise. Death as a result of exposure to sunlight or to desiccation is an example of this essential factor, while the futility of suspending an insoluble so-called disinfectant block in or above a urinal is also obvious.

Although the organism has to be killed by this contact, we are not at liberty to assume that death takes place in one manner only. HgCl_2 does not necessarily kill the same way as $\text{C}_6\text{H}_5\text{OH}$, or again as KMnO_4 . We can make a rough hypothetical classification of disinfectants.

a) Oxidizers.—Here death is caused by direct oxidation and the result in the medium is a loss of organic matter with an increase of CO_2 . The Rideal-Walker figure will not be altered by the addition of silica, except in the case of very unstable oxidizers, i.e., peroxides or hypochlorous acid, where the presence of a finely divided substance catalytically hastens the liberation of free oxygen. Most disinfectants of this class exhibit complications due to (*b*).

b) Ionic poisoning.—H. Crooks (at R.S. Soirée, June 14, 1911) has shown that metals have a germicidal action within a measurable range of their apparent free surface which can be attributable only to the ions of the metals going into solution due to their electrolytic solution pressures. The germicidal value is not necessarily proportional to the electrolytic solution pressures, since the germicidal value of each ion is not necessarily the same. Hence adding silica, etc., and inorganic matter will not decrease the value; adding certain organic matter, i.e., matter that will be affected by the ion, will lower the value. Adding organic matter that is not affected

by the ion, but which acts only as a diluent and increases the ionization will probably alter the germicidal value favorably. HgCl_2 , $\text{Hg}(\text{CN})_2$, AgF , etc., are probable examples of this class. To this class we may add all substances that pass into the system of the body of the organism whether as ions or not, since Kahlenburg has shown that all reactions are not necessarily ionic.

c) *The emulsoid class*.—This is mainly exhibited in the tar derivative disinfectants. Here adsorption on the surface of the body takes place and causes death by altering that surface either by subsequent combination with the tissue or formation of a solid solution, or by purely surface tension effects with formation of a skin which alter the osmotic pressure of the membrane. Methylene blue is observed to stain the skin in this manner and after a time the organism dies. Methylene blue is therefore a disinfectant and can be taken as a type of the surface action class. Here the addition of foreign matter will in general alter the Rideal-Walker figure whether the matter is organic or inorganic.

d) *Ultraviolet light*.—Action unknown.

e) *Heat*.—Complicated by increased oxidation and other chemical changes.

It must be understood that possibly no disinfectant is limited in its action to any one of these arbitrary divisions. Even if the organism were a constant factor it would indeed be strange if such a variety of actions followed a unimolecular law. It is extremely doubtful if any of them agree with that hypothetical reasoning in practice, while they certainly do not in theory.

Another point that has attracted a great deal of attention of late years is the influence of foreign matter on the germicidal value. Foreign matter may be either inorganic or organic, soluble, colloidal, gelatinous, or quite insoluble and massive (opposed to colloidal), of known composition or of unknown structure and variable composition. The addition of such matter may influence both the velocity of disinfection and also the actual amount of useful work a definite quantity of disinfectant can do. It is our duty from a practical standpoint to investigate whether any useful end will be attained by adding any foreign matter when the Rideal-Walker test is performed.

Apart from the practical utility of the addition, there is this technical disadvantage to the process. Foreign matter, if it affects the Rideal-Walker coefficient, generally lowers it. Sommerville and Walker have shown the lowering of value of a number of disinfectants when tested in the presence of such substances as urine, blood, gelatin, starch, etc., and in the method suggested by Chick and Martin a general lowering in value is due to this cause. Let us consider two disinfectants A and B and suppose them to have Rideal-Walker coefficients of 23 and 15 respectively. The Chick and Martin feces method might come out at 5.9 and 5.8; it is clear that a variation of one unit in determination will affect the second series a great deal more than in the first lot. It is known that no two determinations come out quite the same, and the reason for this has been shown above when dealing with the life factor. We would consequently be wiser to accept the larger figures than the smaller ones, as a mere precaution of safety.

The addition of foreign matter, either organic or inorganic, of unknown and variable composition, such as feces, blood, milk, urine, etc., is therefore not to be advised. Even in the Lancet method slight variations in the figure may easily be obtained unwittingly in a well appointed laboratory from which reliable figures usually come, since as the composition alters so does the figure, and although according to this technic the alteration is not great, differentiation between any two disinfectants is no longer possible with guaranteed certainty.

We have discussed the possible methods of disinfection and apparently we can reduce them to the following independent reactions which may take place at the same time, one after the other, or one with another. Observing the types in slightly more detail we see that:

Oxidation of organic matter depends upon the structure of the substance attacked and on the intensity of the oxidation. Hence adding any definite oxidizable matter only gives a figure for that single substance and not for any other; e.g., the oxidation of gun-powder is not comparable numerically to that of mercury but both are oxidation changes and are consequently placed in the same category in physics and chemistry. If the matter is not oxidizable

then there may be catalytic surface decomposition. This depends on the area of the surface of the substance added and probably on its state of division resulting in the liberation of oxygen without necessarily doing effective work. The stability of the disinfectant is, however, assumed if one is bought having a guaranteed Rideal-Walker figure, since if the Rideal-Walker is taken just before use it must have its original value, otherwise the guaranty is useless.

Ionic poisoning.—Here death ensues presumably just as it does in the human organism, by some type of chemical action between the disinfectant and some part of the organism's metabolism, which results in the death of the organism, possibly without such further change as occurs in the oxidation class. It is possible, however, that further action does take place even after death. This would almost certainly occur if the cell wall, for instance, were attacked, for the organism would probably die when only a small part had been destroyed or altered, while the attack would still go on after death. If, however, an important enzyme only were attacked the organism would die when the enzyme was all destroyed and no further change would result. Adding inorganic matter such as silica alumina (colloidal) would not affect the figure to a large degree, although ionic precipitation of the colloid would probably take some of the disinfectant with it. The organic matter that one may add may be affected by the ion or it may not; if the organic matter is not colloidal the reaction will probably take place fairly slowly, as is usual with most organic reactions, and consequently very unreliable and fluctuating results will be arrived at.

Emulsoid class.—Under this class we include all those disinfectants that act by external application only. As we have seen, death may ensue either by alteration of the osmotic pressure, possibly the case with alcohol, by combination with the membrane, by the formation of a solid solution with the membrane, or by being adsorbed by purely surface tension effects. Now it is in this class of disinfectants that most of the velocity work has been done, and it is especially to suit this division that the proposed modifications of the Rideal-Walker method have been proposed. From Chick's work it appears that adsorption certainly does occur in

certain cases. Now inorganic substances exhibit this property also, e.g., charcoal. Travers has shown that the physical nature of the charcoal has a great effect on the adsorption coefficient. Adding charcoal, etc., is consequently similar to adding more bacteria, with the advantage that the charcoal can be made slightly more homogeneous in structure than the bacteria and this lends itself better to the measurement of velocity. We must not consequently give up performing the Rideal-Walker test with bacteria and use charcoal instead for this restricted class of disinfectant, first, because it is bacteria we wish to deal with in practice, and second, because we are not certain that the process is a pure adsorptive one and that toxic influence does not play a part in the proceedings according to our second class. It has been shown that if

x = quantity of disinfectant adsorbed
 a = area of adsorbing surface
 c = concentration of disinfectant

$\frac{x}{a} = KC^n$ where k and n are constants for the system. a is usually measured by the weight of the substance taken.

If, however, we are dealing with cocci, $W \propto r^3$, assuming the cocci homogeneous (which of course they are not), while $a \propto r^2 \therefore a \propto W^{\frac{2}{3}}$. Now this is the equilibrium saturation even if death is due to adsorption only. It has not been shown, however, that microorganisms do not die before saturation is complete, and consequently it may be that a Rideal-Walker with organisms will have a higher value than a modified Rideal-Walker with charcoal in which the end point is measured by the presence of free disinfectant in the solution.

From the above we have seen that we cannot advantageously add matter that has a peculiar chemical structure. We must consequently add matter that remains indifferent to all disinfectants from a chemical standpoint. In its physical aspect, however, the mechanism of a few disinfectants resembles at least in the final stages of the process that one known as adsorption. This we can measure with alumina or, if strong oxidizers are excluded, with standard silk.

As we have seen above, adsorption may not be the only factor concerned, so that a high value for the ratio $\frac{x}{a}$ does not necessarily indicate a high Rideal-Walker, and the reverse is certainly not the case.

Anderson and McClintic¹ suggest modifications of the Rideal-Walker technic without materially altering the results. They prefer *B. typhosus* as the test organism and also admit the desirability of not adding any organic matter in suspension during the duration of the test. The modifications are of only a minor character and are (1) raising the standard temperature to 20° C; (2) fixing the proportion of culture to disinfectant to 0.1 c.c. in 5 c.c. of the disinfectant dilution; (3) taking the mean figure between the strength and time coefficients in a 15 minutes test instead of only one ratio falling within such range.

These changes might easily be accepted by all workers were it not for the fact that the original method is now so widely used in England, India, and her colonies, and we would suggest that at the International Congress in Hygiene, meeting in the United States in 1912, the matter might be finally settled. The temperature 20° C. is somewhat high for general disinfection in northern latitudes and it is as easy to work at the original temperature limits of 16°–18° C. in summer weather as to raise the temperature of the room or reacting bath during winter testing. The general effect of the higher temperature is to give figures which show that disinfection has proceeded more rapidly and that disinfectants have greater germicidal properties at the higher temperatures, so that a greater margin of safety must be allowed when practical disinfection in cool weather is required.

In altering the proportion of culture to disinfectant, care should be taken that the gauge of the wire in the loops used should be sufficiently thick to be rigid and at the same time admit of rapid cooling after sterilization, unless a series of loops as suggested are employed. At the same time accurate results are now certain when one loop only is used in a test, as no two loops can deliver mathematically exact doses.

¹ *Jour. Infect. Dis.*, 1911, 8, p. 1.

As to the adoption of a mean figure instead of the lowest ratio in a harmonious curve, this figure will as a rule be higher than the figure at $2\frac{1}{2}$ minutes and smaller than that at $12\frac{1}{2}$ or 15 minutes. As the aim of disinfection is to insure death in the minimum time, one would be inclined to select the disinfectant which gives the highest figure at the $2\frac{1}{2}$ minutes' interval. The figure obtained at $7\frac{1}{2}$ minutes is very close, if not identical, with that obtained from the mean between the maximum and minimum times included in the testing period.