

A THEORY OF THE MECHANISM OF DISINFECTION,
HEMOLYSIS, AND SIMILAR PROCESSES.

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The apparent course of such processes as hemolysis is determined by the rate of change in the number of living cells which have undergone some definite alteration, such as laking or loss of viability. A great deal of confusion has arisen from the attempts of various investigators to deduce from the observed course of disinfection and hemolysis the nature of their fundamental reactions. Processes of this kind have often been looked upon as due to reactions of a monomolecular type, solely because of a superficial resemblance between the curves expressing the rate of progress of the two phenomena.

This paper is a critical discussion of the part played by the physicochemical process or group of processes leading to death, laking, and similar effects in determining progressive changes in the number of individual cells succumbing in successive units of time to the action of the deleterious agent. These physicochemical processes in the protoplasm may, for the sake of brevity, be termed the "fundamental reaction;" by the "course of the process" we shall understand the time curve of any process like hemolysis or disinfection. The subject is treated in four sections dealing with (1) the evidence that the degree of hemolysis is determined by the number of cells which are laked, and that this depends on the fact that individual cells possess different degrees of resistance; (2) the influence taken singly and in combination, of changes in the variation curve of resistance and of progressive changes in the velocity of the fundamental reaction; (3) the hypotheses advanced to account for the observed course of disinfection, hemolysis, and like processes; (4) the interpretation and significance of the time curves of such processes.

I.

If erythrocytes are suspended in an indifferent medium and subjected to a brief radiation from a mercury vapor arc in quartz, or if they are suspended in an appropriate dilution of serum acting in conjunction with a specific antibody, there ensues a gradual liberation of hemoglobin. In both cases this process begins at a rather slow rate which gradually increases, passes through a maximum, and then gradually falls off until it becomes comparable with the rate of spontaneous laking. (See Table I.)

TABLE I.

Observed Course of Hemolysis; 100 Per Cent Signifies Completion.

10 min. exposure to Hg. arc in quartz.		Specific serum hemolysis.						
		Experiment 26, complement 0.2 per cent.			Experiment 20, com- plement 0.67 per cent.		Experiment 20, com- plement 0.23 per cent.	
Time after radiation.	Colorimetric estimation.	Time.	Color.	Cell count.	Time.	Color.	Time.	Color.
<i>min.</i>	<i>per cent</i>	<i>min.</i>	<i>per cent</i>	<i>per cent</i>	<i>min.</i>	<i>per cent</i>	<i>min.</i>	<i>per cent</i>
5	1	0	0	0	0	0	0	0
12	2	3	3	—	10	65	30	5
19	3	6	5	12	20	94	60	28
37	5	10	10	—	40	100	120	38
55	12	14	21	45			210	45
88	29	20	30	—				
145	55	28	56	55				
205	72	41	67	73				
295	87	56	74	—				
660	100	85	81	79				
		112	81	—				
		155	82	82				
		1,320	100	100				

Plotting as ordinates the amount of hemoglobin liberated, and as abscissæ the times of sampling, one obtains asymmetrical sigmoid curves such as those shown in Fig. 1. The gradual retardation and final apparent equilibrium is either due to exhaustion or inactivation of the lytic agent, or if the concentration of the lytic agent (photo-product or serum) is increased above that necessary to produce complete hemolysis in a few hours, the process ceases because of the ex-

haustion of the supply of cells. (In the latter case we may conceive that the fundamental reaction is still proceeding at a relatively rapid rate even in the most resistant cells at the time when they succumb.)

Enumeration of the red blood cells visible in a given volume of suspension at various stages of hemolysis shows that the amount of hemoglobin liberated is very nearly proportional to the number of cells which have lost their pigment (Table I and Fig. 1). Handovsky¹ finds a similar parallelism between the disappearance of cells and the liberation of hemoglobin when erythrocytes are partially hemolyzed by saponin in low concentrations.

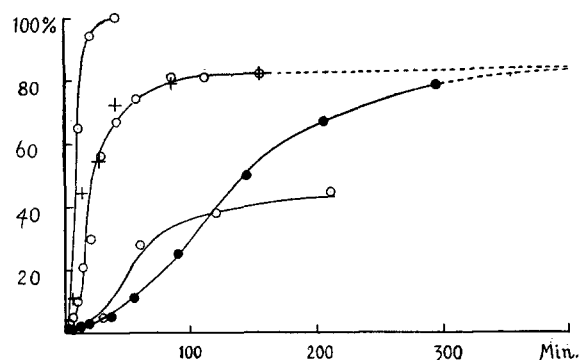


FIG. 1. The time curve of hemolysis produced by ultra-violet radiation (solid circles), and by various dilutions of complement acting on specifically sensitized cells (open circles); the degree of hemolysis was colorimetrically determined. The crosses represent the degree of hemolysis as determined by cell counts during the course of the process represented by the adjoining curve. The ordinates represent the amount of hemolysis in per cent of completion, and the abscissæ, time in minutes.

The course of hemolysis depends therefore on the relative number of red blood cells having in different degrees the power to resist the action of the lytic agent. For since the amount of hemoglobin liberated is always proportional to the number of erythrocytes which have disappeared we must regard the observed course of hemolysis as the summation of the laking of individual cells at varying times after they are subjected to the condition leading to hemolysis. A few relatively

¹ Handovsky, H., *Arch. exp. Path. u. Pharmacol.*, 1912, lxi, 412.

fragile cells are laked almost immediately, the resistant ones survive for many hours, but most of the cells succumb during an intermediate period when the observed rate of hemolysis is at a maximum. When hemoglobin finally begins to diffuse from a given erythrocyte, the process is so quickly completed that it may ordinarily be regarded as instantaneous. It is obvious that an increase in the relative number of, for example, the more fragile cells would accelerate the earlier stages of hemoglobin liberation, while a decrease would produce a corresponding retardation.

II.

There are two ways of expressing graphically the progress of hemolysis: the time curve or course, whose ordinates are proportional to the total number of cells laked; and the rate curve, whose ordinates are proportional to the number of individuals laking in a unit of time. The former is the more frequently found in hemolytic studies, while the latter is the "mortality curve" of vital statistics. If one of these curves is known the other may be found by graphical methods. For instance, let us suppose that we have only the mortality curve: the ordinates of the time curve represent the total number of individuals having insufficient resistance to survive beyond the indicated time; the area enclosed by the mortality curve corresponds to the total number of individuals; the area enclosed by the ordinate at any point on the x axis and the part of the mortality curve to the left of the ordinate, is proportional to the number of cells having less than the indicated resistance, and is therefore also proportional to the corresponding ordinate of the time curve. If the mortality curve is plotted on suitable coordinate paper these areas may easily be estimated with sufficient accuracy, ordinates proportional to them erected at the corresponding points on the x axis, and their tops connected by a smoothed curve. This will be the time curve, and, since the process of finding it is a process of integration, it is at the same time an integral curve.

If we have only the time curve, or integral, it is easy to see that the rate of hemolysis, that is the number of cells laking in a unit of time, is represented by the slope of the time curve; the steeper the time

curve, the more individuals are succumbing per minute. The ordinates of the mortality curve are then at any time proportional to the steepness of the time curve. The slope or steepness is best found by plotting the time curve on suitable coordinate paper, drawing straight lines tangent to it at several points, and counting the number of squares passed over vertically by such lines for a given number passed over horizontally. This ratio is the trigonometric tangent of the slope of the time curve. If we draw ordinates proportional to these ratios at corresponding points on the x axis, and connect the tops of these ordinates by a smooth curve, we shall obtain the rate or mortality curve. Since the process by which we have gotten the rate curve is a graphic method of differentiation, we may consider the curve to be the differential of the time or integral curve.²

Under certain conditions the mortality curve may be identical with the variation or frequency curve of individual resistance. In the following paragraphs the relationship between the time curve and the variation curve is considered, starting with the simplest imaginable conditions, then varying the frequency curve alone, the course of the fundamental reaction alone, and finally both together.

If the rate of hemolysis is uniform, its time curve would be a sloping straight line (the integral curve, a , Fig. 2), while since its tangent or slope is the same at every point, the differential or mortality curve would be a straight line parallel to the axis of the abscissæ (b , Fig. 2); this condition could be expressed by the differential equation

$$\frac{dn}{dt} = k$$

where n is the number of cells, t the time, and k is a constant.

If we now assume the cells to be divided into classes differing from each other by one unit of resistance (r) (defining as a unit of resistance the power to resist one unit of fundamental reaction; *e.g.*, the formation of one mol of toxic substance), and further assume that the fundamental reaction is proceeding at a uniform rate, the differential equation may be replaced by the equation of a frequency curve, or

² This relationship has been suggested by Davey (*J. Exp. Zool.*, 1917, xxii, 573), in connection with curves representing the per cent of death occurring among flour beetles (*Tribolium confusum*) at various times after X-radiation.

variation curve in which all classes are equal in respect to number of individuals. This equation is $y = y_0$, where y_0 is the number of individuals in any one of the equal classes which is arbitrarily selected as the mode, and y is the number of individuals having x units of resistance less or more than the mode. It is obvious that in this particular case any class may be the mode. When the equation assumes a different form so that the classes are unequal (as in the ordinary curves found in biological work) and in consequence y varies, y_0 is the maximum ordinate of the variation curve.

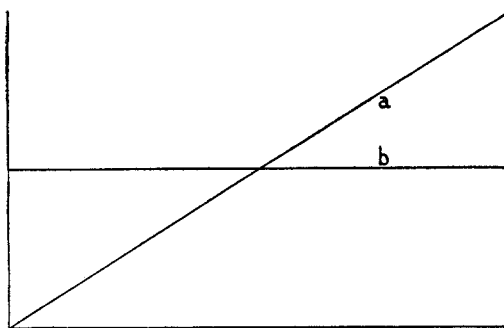


FIG. 2. The relation between the time curve (*a*) of a reaction proceeding at a constant rate, and the curve of the differential equation (*b*). The ordinates represent the extent to which the reaction has proceeded (*a*) or the rate at which it is proceeding (*b*), and the abscissæ represent time.

In order to understand the effect of changes in the shape of such variation curves, that is of changes in the relative number of cells having different degrees of resistance, let us retain the assumption that the fundamental reaction proceeds at a uniform rate; for this allows us to think of the variation curve (abscissæ = resistance) as being at the same time the differential of the time curve (abscissæ = time). Now suppose the variation curve to have the form (*a*, Fig. 3) commonly found in biological statistics, of a "skew frequency curve of limited range" whose equation according to Pearson³ is

$$y = y_0 \left(1 + \frac{x}{x_1}\right)^{kx_1} \left(1 - \frac{x}{x_2}\right)^{kx_2} \quad (1)$$

where k is a constant, and x_1 and x_2 the number of degrees of resist-

³ Pearson, K., *Phil. Tr.*, A, 1895, clxxxvi, 343.

ance less and more, respectively, than the mode, possessed by the most fragile and most resistant classes. Here $x_1 + x_2$ is the total range of resistance of all the cells. A curve of this type (*a*) and its integral, or time curve, (*b*) are shown in Fig. 3; the time curve has a form not unlike that of the curves for the course of hemolysis.

If we suppose, with some investigators, that the resistance of the cells varies around an average from which it does not deviate to an extent sufficient to influence the course of the process, we must consider all the cells to be in a single class with respect to resistance.

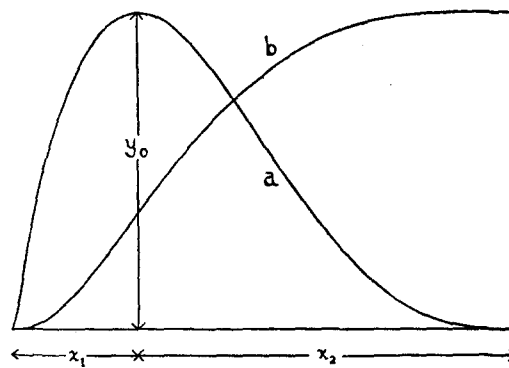


FIG. 3. (*a*) variation curve whose equation is

$$y = y_0 \left(1 + \frac{x}{x_1}\right)^{kx_1} \left(1 - \frac{x}{x_2}\right)^{kx_2}, \text{ where } x_1 = 2, x_2 = 6, k = 0.5.$$

If (*a*) is considered as the curve of a differential equation, (*b*) is the curve of the corresponding integral. The ordinates of (*b*) are proportionate at any position on the x axis (abscissæ) to the area to the left of the ordinate at that position, and under the curve (*a*).

The frequency curve will then be so narrow as to be approximately a straight line normal to the axis of the abscissæ at some point. Its integral, which is the time curve of the process, will follow the axis of abscissæ to this point, and then rise perpendicularly to its ultimate height. In other words, if the process were hemolysis, all the cells would lye at the same instant. This conclusion may be avoided, theoretically at least, by making one of the special assumptions which are discussed below.

As a matter of fact the exact shape of the frequency curve—that is the relative abundance of cells having different degrees of resistance to any given lytic agent—is not of general significance, for it depends on the condition and previous treatment of the animal from which the cells are secured, on the nature of the lysin, etc. For example, Handovsky¹ has shown that during the regeneration of erythrocytes following artificially induced anemia there are two groups of erythrocytes, one of which has a higher average resistance to hemolysis by saponin than the other; differentiation of curves representing the course of the hemolysis of such blood cells gives a bimodal variation curve. The same author has shown that alterations in resistance may appear in opposite senses according to the choice of lytic agents; *e.g.*, the blood cells of dormant bats are less resistant to the action of saponin, but more resistant to that of sodium hydroxide than those of active bats.

We may sum up the influence of variations of resistance by saying that they determine the general shape and points of inflection of the curves expressing the course of such processes, and that, therefore, in the absence of further analysis such curves tell us nothing as to the nature of the fundamental reaction.

Changes in velocity during successive stages of the fundamental reaction will obviously produce corresponding changes in the time required to produce a given degree of hemolysis, and will therefore alter the shape of the time curve. We have seen that when the equation of the variation curve of resistance is of the form

$$y = y_0$$

and the velocity of the fundamental reaction is constant, the time curve of the reaction is identical with that of the process as a whole. A moment's reflection will show that, whatever the course of the fundamental reaction, so long as the variation curve remains of this form the same identity will appear. If for example the fundamental reaction is monomolecular, the course of the process will appear monomolecular.

The relative length of time required for the process to reach any given stage (*i.e.* the abscissa of the time curve for any given ordinate) will be greater for the same initial velocity in the case of a monomo-

lecular reaction than in the case of a reaction proceeding at a constant rate, and the lengths will be still greater for reactions of higher orders.

The relative times, t_1 and t_0 , required for two reactions with the same initial velocity to reach the same stage, when one is monomolecular and the other has a constant velocity, may be easily derived from the equations of the two time curves. The expression is

$$\frac{t_1}{t_0} = \frac{1}{x} \ln \frac{a}{a-x}$$

where \ln is the logarithm to the base e ($2.3026 \log_{10} \frac{a}{a-x}$ may advantageously be used in place of $\ln \frac{a}{a-x}$), and where a is the initial amount of the reacting substance, and x the amount transformed at any given stage; a and x are usually stated in per cent.

If we compare the rates of hemolysis which would result from the action of two fundamental reactions of different orders, we find that the relative time required to reach a given stage will be the same regardless of the variation curve of the cells affected; if the shape of the variation curve is given by some equation other than $y = y_0$ (which gives identical time curves for the fundamental reaction and for the process as a whole), we can still calculate the relative lengths of time required for the process to reach a given stage, according to the order of the fundamental reaction. If the fundamental reaction is monomolecular or of a higher order, we must use as abscissæ distances which are as much greater than those of the time curve when the fundamental reaction has a constant velocity, as is indicated by calculations like that given above. This is most easily done if it is assumed that the hemolysis and the fundamental reaction reach completion simultaneously, but may still be calculated for a known excess or deficiency of the lytic agent. In Fig. 4 are given the courses which would be assumed by a process taking place in a group of cells, the equation of whose variation curve was

$$y = y_0 \left(1 - \frac{x^2}{x_1^2} \right)^{kx_1}$$

if the fundamental reaction were (a) proceeding with a constant velocity; (b) proceeding according to the law of monomolecular

reactions. Here again we find a curve *b* which simulates the observed curves for the course of hemolysis. We shall return to this point later on.

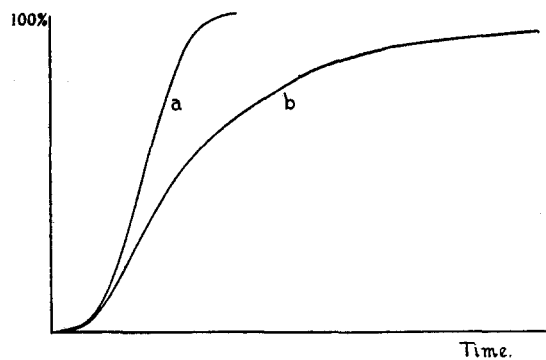


FIG. 4. The time curve of a process occurring in a group of individuals having a variation curve with the equation $y = y_0 \left(1 - \frac{x^2}{x_1^2}\right)^{kx_1}$, if the fundamental reaction proceeds at a uniform velocity (*a*), and if it proceeds according to the law of monomolecular reactions (*b*). Here $x_1 = 1.5$ and $k = 2$. The ordinates represent degree of completion of the process, and the abscissæ, time.

III.

Various investigators have reported that bacteria in the presence of disinfectants die at a rate which is at any given time proportional to the number of organisms then surviving.⁴⁻⁹ This condition would be described by the equation

$$\frac{dn}{dt} = kn, \text{ or } n_t = n_0 e^{-kt}$$

where n_0 and n_t are the number of bacteria at the beginning of the experiment and at t units of time thereafter, and e is the base of the natural system of logarithms. This is the equation of the mono-

⁴ Madsen, T., and Nyman, M., *Z. Hyg.*, 1907, lvii, 388.

⁵ Chick, H., *J. Hyg.*, 1908, viii, 92.

⁶ Chick, *J. Hyg.*, 1910, x, 237.

⁷ Phelps, E. B., *J. Infect. Dis.*, 1911, viii, 27.

⁸ Eijkman, C., *Folia Microbiol.*, 1912, i, 359.

⁹ Reichenbach, H., *Z. Hyg.*, 1911, lxix, 171.

molecular reaction isotherm. Similar relationships have been reported in the case of hemolysis, which is a process in many ways similar to disinfection;¹⁰⁻¹⁴ although in this case a so called "induction period," often of considerable length (Dreyer and his coworkers) intervenes before the hemolysis appears to follow the course of a monomolecular reaction. In both hemolysis and disinfection a large number of single living cells are exposed to the action of an agent which ultimately induces in the cell some change which we can detect; in one case loss of power to reproduce; in the other, loss of a pigment; in both cases there is great variation in the length of time required to bring about the critical change in different cells. Harvey¹⁵ considers the equation for monomolecular reactions to be applicable to the loss of motility suffered by cells of *Chlamydomonas* subjected to the action of hydrochloric acid in great dilution; while Darwin and Blackman, according to Arrhenius,¹⁶ saw the same relationship when they allowed various killing agents to act on seeds. These citations, while by no means complete, will suffice to indicate the wide range of phenomena which have been studied from this point of view.

Some authors, like Dreyer and his coworkers, have not attempted to attribute the apparent analogy of these processes with monomolecular reactions to any single relationship, but some other authors have devoted to this phenomenon a great deal of discussion which does not seem to have been based upon a comprehensive knowledge of the subject, for their attempts to explain the analogy will not bear criticism, and none of their criticisms are entirely satisfactory. The difficulty is largely due to failure to see the necessary consequences of uniformity in resistance, or to disregard of the possible influence of progressive changes in the velocity of the fundamental reaction.

¹⁰ Arrhenius, S., and Madsen, T., *Z. physik. Chem.*, 1903, xliv, 33.

¹¹ Henri, V., *Compt. rend. Soc. biol.*, 1905, lvii, 37.

¹² von Liebermann, L., and von Fenyvessy, B., *Z. Immunitätsforsch., Orig.*, 1912, xii, 417.

¹³ Salomonsen, C. J., and Dreyer, G., *Compt. rend. Acad.*, 1907, cxliv, 999.

¹⁴ Dreyer, G., and Hanssen, O., *Compt. rend. Acad.*, 1907, cxlv, 371.

¹⁵ Harvey, H. W., *Ann. Bot.*, 1909, xxiii, 181.

¹⁶ Arrhenius, S., *Quantitative laws in biological chemistry*, London, 1915, interprets in this way the experiments of Darwin, N., and Blackman, A., *Rep. 78th Meeting, 1908, Brit. Assn. Adv. Sc.*, 1909, 902.

Madsen and Nyman,⁴ who were the first to notice the analogy, recognized the fact that variability among the cells was a factor to be reckoned with, but appear nevertheless to have regarded their curves as expressions of the average rate of change in the individual cells. We have seen that it is impossible, without special assumptions, to account for the phenomenon on this basis. Miss Chick,⁵ working independently, secured data like those of Madsen and Nyman, but states explicitly that the monomolecular reaction formula is applicable to the process, "one reagent being represented by the disinfectant" which being present in excess may be regarded as having a constant concentration, "and the second by the protoplasm of the bacterium;" she amplifies this statement by making the supposition that the bacteria undergo rapid cyclic variations in their ability to react with the disinfectant. Phelps⁷ in developing Miss Chick's method for standardizing disinfectants, adopts the same explanation. Arrhenius¹⁶ says, "there is no doubt that the different cells in a sample of bacteria or red blood-corpuscles possess a different power of resistance to deleterious substances," but that "the different lifetime of the different bacteria does not, therefore, depend in a sensible degree on their different ability to resist the destructive action of the poison," and accepts Chick's explanation, as does Eijkman,⁸ at least in the case of certain bacteria.

The acceptance of such an explanation makes it necessary to assume that loss of viability, like the breaking up of a single molecule of saccharose during inversion, takes place in a single step; in other words, that the disinfectant cannot have any cumulative effect on the viability of individual cells. If the loss of viability occurred in two or more steps, some or all of the cells surviving at any time during the process would be "partially dead," and a greater proportion of them would succumb in any given interval of time than would have done so during the same interval at the beginning of the process when all of the cells were entirely unaffected. In other words the per cent death would increase during the process instead of remaining constant as demanded by the law of monomolecular reactions.

This assumption that death occurs at a bound, as it were, is surprisingly at variance with the usual conception of vital processes. It seems to necessitate that we regard a living cell as being dynamically

comparable to a molecule. For this reason Robertson¹⁷ has offered another explanation for the apparent exponential decrease in the number of surviving cells; *i.e.*, for the apparent applicability of the law of monomolecular reactions. His explanation assumes the collisions with the disinfectant molecules to be distributed fortuitously among the different individuals of a homogeneous group of cells. Since it would be out of place to consider here the details of his mathematical proof, it must suffice to point out that Robertson's quantity x , "the number of units of the underlying change," must apparently be at one and the same time a constant, and an exponential function of time. This impossible assumption is the basis of Robertson's whole proof. Other mathematical inconsistencies occur, but are of relatively little consequence.

In a subsequent paper⁶ Miss Chick modifies her original theory by assuming that it is the protein molecules of the bacterial protoplasm, which, like the sugar molecules during hydrolysis, undergo the cyclic changes in energy content, upon which depend their ability to react. The concentration of these protein molecules at any moment would then, according to Miss Chick, determine the rate of death of the bacteria at that moment. We have already seen that under these conditions the cells of a group of uniformly resistant individuals, such as Miss Chick postulates, would all die at one time. If the course of disinfection is to parallel that of the reaction, the diametrically opposite condition must prevail; namely, one in which the cells are equally distributed among all the possible classes of resistance; in other words, the equation of the variation curve must be $y = y_0$.

Von Liebermann and von Fenyvessy¹² explained the hemolytic "monomolecular curve" on the basis of the probable rate of exit of hemoglobin from the individual cell, from which the pigment might be supposed to diffuse at a rate proportional to the difference between the intra- and extracellular hemoglobin concentrations. This explanation is evidently in conflict with the observed progressive decrease in the number of intact erythrocytes, and with the fact reported by Dienes¹⁸ that there is a nearly constant ratio between the hemoglobin

¹⁷ Robertson, T. B., *J. Hyg.*, 1914, xiv, 143.

¹⁸ Dienes, L., *Biochem. Z.*, 1911, xxxiii, 268.

content and the dry weight of the residual cells at various stages of hemolysis.

Since none of the explanations given above is satisfactory let us see whether the experimental facts necessitate the assumption that the equation of the monomolecular reaction is a complete description of the process of disinfection. Examination shows that none of the experiments already referred to include the observations necessary for the study of the first part of the process; the second observation was almost without exception deferred until the process was nearly half complete. It also appears that in many experiments the "initial" number of cells was determined after an appreciable part of the process had occurred, that is after the most fragile cells had already succumbed, and while the reaction was proceeding so rapidly that the slightest errors in the time of sampling would produce great differences in the number of cells recorded.

It is quite impossible to judge correctly the validity of formulas based on such inadequate data. In Fig. 5, I have plotted the points of that one of Chick's curves which shows the best agreement with a monomolecular reaction curve, a similar curve for the number of *Chlamydomonas* cells immobilized at intervals after exposure to dilute hydrochloric acid (Harvey,¹⁵), a curve for normal serum hemolysis (Henri,¹¹), and an original curve (from which the first few observations are omitted) for hemolysis after ultra-violet radiation. In the last two cases hemolysis was estimated colorimetrically. A monomolecular reaction curve is drawn for comparison with these points, and the scales of the abscissæ are so arranged as to make the curves coincide at the point where the effect is half completed. The agreement of the points with the monomolecular reaction curve is about equally close. But in this same figure the curve for the original hemolytic experiment is also drawn with the abscissæ on a larger scale and with the first few observations included. It is obvious that these first few observations are most important. One suspects that had these observers but made sufficient observations during the first part of the process, they would have found that disinfection, killing of seeds, etc., are processes which, like hemolysis, are initially slow and only subsequently attain their maximum velocity. As a matter of fact this is what a number of other workers have found.

Dreyer and his coworkers, for example, found that only after a considerable period of slow laking, did the course of the hemolysis induced by α and β rays of radium and by ultra-violet radiation follow the course of a monomolecular reaction; this initial lag they call, tentatively, a period of induction.

Eijkman⁸ has shown that the shape of the disinfection time curve is peculiar to the particular culture of bacteria used, and to some

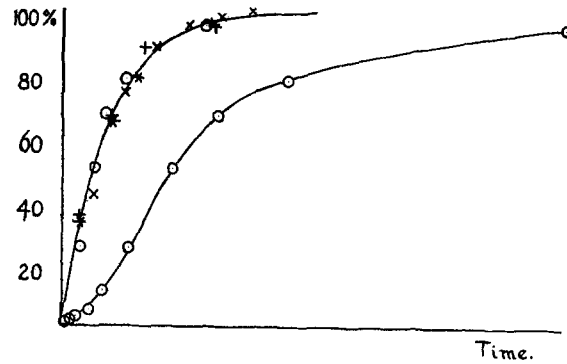


FIG. 5. The upper curve is the monomolecular reaction isotherm, beside which are plotted experimentally determined points for normal serum hemolysis (Henri, asterisks), the immobilization of *Chlamydomonas* by 0.009 per cent HCl (Harvey, diagonal crosses), disinfection (Chick, erect crosses), and hemolysis by ultra-violet radiation (original, circles); the abscissæ were so adjusted in plotting these points as to make their curves coincide with the monomolecular reaction isotherm at 50 per cent. The lower curve represents the course of the same ultra-violet hemolysis, but with the abscissæ plotted on a larger scale, and with the addition of 5 points determined during the first part of the process. The ordinates represent degree of completion of the process, and abscissæ, time.

extent characteristic of the species. Both Eijkman and Reichenbach⁹ have secured disinfection time curves which, even when determinations during the early stages of the process are included, do not diverge very greatly from the exponential or monomolecular type of curve. It is possible to explain such curves either by assuming a monomolecular fundamental reaction and a variation curve of the form $y = y_0$, or by assuming a fundamental reaction proceeding at a uniform rate, and a variation curve of the form

$$y = y_0 e^{-kx} \quad (2)$$

where k is a constant. The latter equation is that of the monomolecular reaction isotherm; but it gives a curve having unlimited range, a condition which makes it undesirable in the case of a limited population; it is better to use a curve of very similar appearance which results if we put $x_1=0$ in equation (1) above. We thus introduce the necessary factor of limited range given by x_2 . The equation, so modified, becomes

$$y = y_0 \left(1 - \frac{x}{x_2}\right)^{kx_2}$$

while if $\frac{x}{x_2}$ is made indefinitely great (*i.e.* if we consider only the first part of a curve with infinite range) it approaches an equation identical with (2) above.

Reichenbach has shown that if we imagine a constant fraction of the bacteria of each generation to lose their power to divide, and suppose that at each successive division there occurs a decrease in the resistance of the dividing organisms, the individuals with the highest resistance, *i.e.* those of the first generation, which have not since undergone division, will be present in the smallest numbers, and that those of succeeding generations (and which have therefore less resistance), will be present in numbers increasing in geometrical proportion. Under certain conditions such a culture would have nearly the type of variation curve necessary to give the "monomolecular" curve of killing; and Reichenbach finds in individual variation a complete explanation of the observed curves of disinfection. This hypothesis is not, however, applicable to the process of hemolysis, and can therefore have no general significance. The agreement of the middle portion of hemolytic and other similar time curves with the monomolecular reaction curve is probably only a coincidence, and is not of fundamental significance.

Yule¹⁹ has shown that it is possible, theoretically at least, to account for a time curve of hemolysis such as that which I have observed, which, rather than a monomolecular curve, is probably characteristic of the other processes here discussed, even if all the cells are assumed to be equally resistant. This involves the assumption that a certain small number, r , of independent changes suffices to cause lysis. Probably in the case of hemolysis r would not greatly exceed 2. If $r = 1$,

¹⁹ Yule, G. U., *J. Roy. Statist. Soc.*, 1910, lxxiii, 26.

Yule's formulas lead to a "monomolecular" curve. It seems more rational to adopt some explanation which takes into account the individual variations in resistance, thus avoiding unnatural assumptions as to the nature of the fundamental reaction.

IV.

The idea that the rate of disinfection is due to variations in resistance is not new; Geppert²⁰ gave expression to it nearly a generation ago. The same idea has since been restated in many forms and in many connections, but always without reference to any influence which might be exerted by the fundamental reaction.

Mioni^{21,22} criticizes Henri's studies¹¹ on hemolysis, because Henri neglected the influence of individual variation; Mioni's experimental evidence is inconclusive, however. Dienes¹⁸ suggested that various degrees of resistance to hemolysis were distributed in accordance with "Quetelet's law" ($y = y_0 e^{-kx}$). Hewlett²³ says, with reference to the hypothesis offered by Miss Chick in her first paper, "While admitting that the disinfection of anthrax spores follows the *course* of a unimolecular reaction, I think it extremely doubtful, to say the least, if the reaction between disinfectant and bacterium *is* a unimolecular reaction." Hewlett's own experiments on the effect of mercuric chloride on the viability of mustard seeds accord with those of Darwin and Blackman,¹⁶ but he says: "It appears to me that only by a wide stretch of the imagination can the interaction of mustard seed and disinfectant be considered as a unimolecular reaction, or a reaction of a higher order, yet it follows approximately the course of the former."

Loeb and Northrop²⁴ also criticize the idea that disinfection is due to a monomolecular reaction. They say Miss Chick "was probably led to such an assumption by the fact that the ascending branch of the mortality curve in her experiments was generally very steep . . . almost a vertical line, thus escaping detection. Hence she noticed usually only the less steep descending

²⁰ Geppert, J., *Berl. klin. Woch.*, 1889, xxvi, 789.

²¹ Mioni, G., *Ann. Inst. Pasteur*, 1905, xix, 84.

²² Mioni, *Compt. rend. Soc. biol.*, 1905, lvii, 485.

²³ Hewlett, R. T., *Lancet*, 1909, i, 889.

²⁴ Loeb, J., and Northrop, J. H., *J. Biol. Chem.*, 1917, xxxii, 103.

branch which could be interpreted as a monomolecular curve for the reason that her experiments lasted only a short time." Miss Chick's published curves were, as a matter of fact, time curves, *i.e.* "integrals" of "mortality" or variation curves, and had she been able to make sufficient observations at the beginning of the process, she might have obtained curves at first horizontal, but very quickly turning downwards. Such a condition would find expression in a mortality curve as an ascending branch rising very steeply from zero to a maximum.

The true explanation of the course of processes like disinfection is undoubtedly a combination of the two extreme views: one attributing the course to variation alone, the other considering it to express the nature of the fundamental reaction alone. Both of these factors exert an extremely important influence. We have seen that it is theoretically possible to relegate variation to a position of unimportance by assuming a variation curve having the form $y = y_0$. Such a distribution of various degrees of resistance is obviously unnatural, and the assumption that it occurs must, in the absence of definite evidence, be considered unwarranted. Its use frequently necessitates the postulation of "latent" or "induction" periods of whose existence we have no further proof. On the other hand we may consider the reaction velocity as constant, and by graphic differentiation obtain not unnatural frequency curves. Perhaps by employing several constants we may even obtain applicable equations; but the constants will have no physical meaning, and the equations no general significance.

The reaction velocity, as a moment's consideration will show, must ordinarily decrease as the process goes on; for "sub-minimal" amounts of the toxic substances carry the process to partial completion, and whatever the final "equilibrium" may be, it is gradually attained; the process does not abruptly cease.²⁵

²⁵ Curves drawn through points expressing the amount of toxic agent required to produce various degrees of completion of such processes might be regarded as "integrals" of the frequency curves of resistance. In practice, however, this reasoning is applicable only to such *in vivo* experiments as determination of therapeutic efficiency or toxicity of drugs, radiation, etc. *In vitro*, factors such as bacterial contamination, autolysis, cell division, or starvation are likely to supervene, and distort the observed curve.

Should we not then conclude that the course of processes like disinfection is, like b in Fig. 4, the result of the simultaneous operation of two factors: the frequency curve of variation in individual resistance, which may be different for each group of cells and each toxic agent; and the course of the fundamental reaction, which usually proceeds with a velocity diminishing during the experiment at a rate dependent on the particular conditions prevailing? We must also bear in mind that what we have supposed to be the fundamental reaction may be the end result of a complex series of interrelated or "catenary" reactions. If some one link in this chain of events is a change which, from the beginning to the end of the process, is so slow as to govern the rate of the whole series, which taken together is regarded as the fundamental reaction, then, and only then, will orderly laws describe the course of the latter.

This conception does indeed render a solution of the problem much more difficult than it once seemed, but not necessarily unattainable. A proper understanding of this "group experiment," as we might perhaps call the widely employed type of which disinfection is but one example, should lead to better interpretation of the phenomena themselves, and a far deeper insight into the fundamental life processes to which they are due.

CONCLUSIONS.

1. The course of such processes as hemolysis is very largely dependent upon variations in resistance among the different individuals, and secondarily upon the course of the fundamental reaction.
2. The fundamental reaction may be either a simple process, or the expression of a complex series of changes whose rate is at all times governed by that of the slowest of the series. This might perhaps be regarded as another expression of the so called "Law of the minimum."
3. Unnatural assumptions would be requisite for the explanation of a resemblance between the course of such processes in general and that of a monomolecular reaction.
4. The supposition that such a general resemblance exists is not supported by the available evidence.

5. The independent determination of either the nature of the fundamental reaction, or the type of the variation curve for the particular case under observation, will further our knowledge of the nature of such processes and lead to a far deeper insight into the nature and reactions of living matter.