ON THE CONDITIONS UNDER WHICH DISCONTINUOUS EVENTS MAY BE EMPLOYED AS A MEASURE OF CONTINUOUS PROCESSES, WITH ESPECIAL REFERENCE TO THE KILLING OF BACTERIA BY DISINFECTANTS.

By T. BRAILSFORD ROBERTSON.

(*From the Rudolph Spreckels Physiological Laboratory of the University of California.*)

It very frequently happens in the investigation of life-phenomena that the uninterrupted progress of underlying processes is evidenced to our senses by a series of intermittent events. Thus the underlying and undoubtedly continuous processes which determine the heart-beat are evidenced to our senses by a series of intermittent pulsations; the effects of previous sensory stimulation upon our central nervous system are usually recognised by the recollection of a series of images which appear to us to be discrete and unitary in character; the continuous effects of light upon non-sessile heliotropic organisms are evidenced by their separate and unequal movement towards the source of illumination, and the action of a disinfectant upon bacteria is evidenced by a series of deaths, each individual death constituting an indivisible unit.

No especial consideration is required in order to appreciate the fact that the frequency with which any of the above discontinuous events occur affords some sort of indication of the extent to which the underlying and determining processes are taking place. It is not by any means so obvious, however, that the number of these discontinuous events is a reliable *quantitative* measure of the progress of the underlying processes. If indeed it be so, then a knowledge of that fact and of the limiting conditions under which this method of measurement may be

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safely applied must be of paramount importance to the biologist, since the method opens up to him the possibility of a quantitative estimation of innumerable life-processes which would otherwise be inaccessible to measurement.

In a series of communications to this Journal H. Chick (1908–1912)[1] has confirmed and very greatly extended the observation of Madsen and Nyman (1907)[2] that if a uniform culture of bacteria be exposed for varying periods of time to the action of a relatively large amount of disinfectant (so that the concentration of disinfectant does not appreciably alter throughout the process), the relationship between the number of bacteria killed and the time of exposure is that which is characteristic of a mono-molecular reaction, that is, of a reaction in which only one molecular species is undergoing appreciable changes in concentration. They interpret[3] this fact to mean that the underlying process which determines the death of the bacteria (*e.g.* combination of the disinfectant with some protein within the bacteria) is a chemical reaction involving a concentration-change in one molecular species. It is obvious that the validity of this conclusion depends upon the validity of employing the discontinuous events afforded by the deaths of the bacteria as a measure of the extent of the continuous underlying chemical changes *within* the bacteria.

G. Udny Yule (1910)[4] has pointed out that the death-rate in these experiments *cannot be selective*, in other words that the successive deaths cannot be attributed to inequalities in the susceptibility of the bacteria, for otherwise the percentage-mortality would *decrease* with time as the weaklings were weeded out, whereas the results above-quoted show that the percentage death-rate is *constant*, just as the percentage of change is constant in a mono-molecular reaction. On the other hand he finds difficulty in accounting for the results on the supposition that the action of the disinfectant upon the bacteria is gradual and cumulative, as one must necessarily assume it to be if it really consists in a chemical process.

Starting with the assumption that the chance ($=p$) of an "unfavourable" change occurring in any one of the bacteria (such as the combination of a molecule of disinfectant with a molecule of the proteins which it contains) is constant for all periods of exposure. Yule

[1] H. Chick. *Journal of Hygiene*, (1908) VIII. 92; (1910) X. 237; (1912) XII. 414.

[2] Madsen and Nyman (1907). *Zeitschr. f. Hyg.* LVII. 388.

[3] As far as we know Madsen and Nyman offered no interpretation of the facts they brought forward. (Ed.)

[4] G. Udny Yule (1910). *Journal of the Royal Statistical Society*, LXXIII. 26.

finds that the law of mortality observed by Chick cannot be accounted for except upon the supposition that a *single* "unfavourable change" is fatal,—a supposition so inherently improbable that it may be dismissed without further consideration. If, however, we examine the actual implications of Yule's fundamental assumption that p is constant, we find that it is equivalent to assuming *that the underlying process which determines the death of the bacteria is not a chemical process*, for it is a fundamental characteristic of the time-relations in chemical processes that the frequency with which units of change occur (and therefore the probability of their occurrence) does not remain constant as a reaction proceeds but, on the contrary, varies in accordance with laws which are definite and dependent upon the number of molecules which participate in undergoing a unit of change. Thus, if the reaction be mono-molecular, the probability ($=x$) of a unit of change taking place in time $=t$ is given by:—

$$\log \frac{A}{A-x} = \kappa t$$

where A and κ are constants, and the probability of a unit of change taking place in a *unit* of time is given by the value of $\frac{dx}{dt}$ in the equation:—

$$\frac{dx}{dt} = \kappa (A-x),$$

the constant A expressing the initial mass of material subject to change.

Hence, what Yule actually proves is that *if the underlying process which determines the death of the bacteria is not a chemical process, or at least a process of which the velocity varies as it proceeds, then the quantitative results obtained by Chick are inexplicable.*

The question still remains, however, to what extent we may rely upon the quantitative results obtained by Chick and by Madsen and Nyman as *quantitatively defining* the processes which underlie disinfection. This question may be answered in the following way:—

Let x be the number of units of the underlying change which has taken place in a given time t in all of the bacteria taken together. These units of change will be distributed fortuitously among the different bacteria, so that in a certain number of them 0 units of change will have occurred, in others 1 unit, in others 2 units, and so forth. The fortuitous character of the distribution arises from the fact that in order that a unit of change may occur in any one of the bacteria it must first of all receive (collide with) a molecule of the disinfectant, and the collisions between disinfectant-molecules and bacteria are necessarily fortuitous.

If there be N bacteria in all which are exposed to the action of n molecules of disinfectant, *and they do not cease at any time to be exposed to the action of the disinfectant*[1], then there are n ways in which a collision may occur with any one of the bacteria, and the chance of any given *one* of the bacteria receiving a single collision is $\frac{1}{N}$ and the chance of its failing to do so is $\frac{N-1}{N}$. Hence the number of bacteria which will have undergone $0, 1, 2, \ldots\ldots r, \ldots\ldots$ units of change ("successes") when the total number of units of change which have occurred is x will be given by the successive terms of the following series[2], *provided that n does not appreciably alter during the course of the reaction*[3]:—

$$x\left\{\left(\frac{N-1}{N}\right)^n + n\left(\frac{N-1}{N}\right)^{n-1}\left(\frac{1}{N}\right) + \frac{n(n-1)}{1.2}\left(\frac{N-1}{N}\right)^{n-2}\left(\frac{1}{N}\right)^2 + \ldots\ldots \right.$$
$$\left. + \frac{n(n-1)\ldots\ldots(n-r+1)}{\underline{|r}}\left(\frac{N-1}{N}\right)^{n-r}\left(\frac{1}{N}\right)^r + \ldots\ldots + \left(\frac{1}{N}\right)^n\right\}.$$

Let us suppose that the death of *any* given one of the bacteria occurs when r units of change have taken place within it, that is, that the "susceptibility" of every individual of the culture is the same. Then the number of bacteria which will have died at time t after the exposure began will be the sum of the least $n-r+1$ terms of the above series, hence we have:—

$$y = x\left\{\frac{n(n-1)\ldots\ldots(n-r+1)}{\underline{|r}}\left(\frac{N-1}{N}\right)^{n-r}\left(\frac{1}{N}\right)^r + \ \ldots + \left(\frac{1}{N}\right)^n\right\}.$$

If N be very large compared with unity, as it was in the experiments

[1] In other words, that the underlying change does not cease with the death of the bacteria, *i.e.* that the bacteria remain uniformly suspended in the solution of the disinfectant even after death. This condition was fulfilled in the experiments under consideration. If the bacteria were for any reason removed from the sphere of action of the disinfectant at death, for instance by falling out of suspension, then N at any moment would be equal to $N_1 - y$, where N_1 was the initial number of bacteria exposed to the disinfectant. In other words, unless y were very small in comparison with N_1, N would vary with the time, and the time-relations observed by Madsen and Nyman and by Chick could not be obtained.

[2] Cf. G. Udny Yule (1911), *An Introduction to the Theory of Statistics*, London 1911, p. 289.

[3] Provided, that is, that the quantity of disinfectant used up in killing all of the bacteria was evanescently small in comparison with the total quantity of disinfectant employed. This condition was obviously fulfilled in the experiments to which I have referred.

under consideration, then $\frac{N-1}{N}$ becomes equal to unity and the above equation may be somewhat simplified and written as follows:—

$$y = x\left(\frac{1}{N}\right)^r \left\{\frac{n(n-1)\ldots\ldots(n-r+1)}{\underline{|r}} + \frac{n(n-1)\ldots\ldots(n-r+1)(n-r)}{\underline{|r+1}}\left(\frac{1}{N}\right) + \ldots\ldots + \left(\frac{1}{N}\right)^{n-r}\right\}.$$

In any case, whatever be the absolute magnitude of N, the sum of the terms enclosed within the brackets will obviously be the same at all stages of the reaction, since it depends only upon the magnitudes of n, N, and r which, it has been assumed, do not alter during the progress of the reaction. Calling this sum μ, we have:—

$$y = \mu x.$$

Then if $x = f(t)$ is the relationship between x and the time of exposure:—

$$y = \mu f(t).$$

For instance, if $f(t)$ is of such a nature that:—

$$\log \frac{A}{A - x} = \kappa t,$$

i.e. if the reaction is "mono-molecular," then, substituting from the above equations, we have:—

$$\log \frac{\frac{N}{\mu}}{\frac{N}{\mu} - \frac{y}{\mu}} = \kappa t,$$

whence:—

$$\log \frac{N}{N - y} = \kappa t,$$

which is the law of mortality which has been experimentally verified by the above-quoted observers for a uniform culture of bacteria.

It is obvious that these deductions are of a perfectly general character, and that we may employ N in the above equations to denote "total number of heliotropic organisms exposed to light," "total number of muscle-fibres stimulated," etc., just as we have employed it to denote "total number of bacteria exposed to disinfectant." Similarly we may employ y to denote "number of reacting organisms," "number of contracting fibres," etc., and n to denote the corresponding quantitative conditions defining the *environment* to which the reacting units are exposed. In every such case y (= number of reacting individuals) will be proportional to x (= extent of underlying change in all of the individuals taken together) provided that N and n be constant throughout the change, and that the extent of the change within an individual necessary to cause it to display the given "signal" (*i.e.* death, orientation towards a source of light, contraction, etc.) is also constant throughout the process, and very nearly the same for all of the individuals.

Summary.

It is shown that provided the total number of individuals exposed to a constant environment which is inducing change within them be constant, and the number of units of change which must take place within any given individual in order to cause a given event be also constant, then the number of these events is a quantitative measure of the extent of the change in all of the individuals taken together.

From this it follows that the results of Madsen and Nyman and of Chick may legitimately be regarded as proving that the process which underlies disinfection obeys the time-relations and other characteristics of a mono-molecular chemical reaction.