very interesting subject, bringing to light a most interesting palaeontological result and ably conducted, might have brought us still nearer to the truth if the Linnean method had been used in making the descriptions.

This method indeed asks much of the investigator's time and energy and the use of it can only be learnt by patient study. But we mean to say, that at some future time a botanist of Mrs. STOPES' power will not be satisfied with descriptions of anatomical structures made without the use of the Linnean principles of micrography.

Groningen, Oct. 21th 1912.

Bacteriology. -- "*On the reaction velocity of Micro-organisms*". By Prof. C. EIJKMAN.

(Communicated in the meeting of September 28, 1912).

I. *Velocity of disinfection.*

Micro-organisms have been the object of various researches as regards the velocity of their reaction, when exposed to external agents. From the experimental evidence brought forward it appeared, that considerable differences exist between individuals of the same species, of the same stock, nay of the same culture: they do not react all about at the same time, but the reaction proceeds in an orderly manner.

It is especially the orderly progress of disinfection of bacteria, under the influence of germicidal agents, either chemical or thermal, which, in virtue of its vital importance for theory as well as for practice, has recently been studied by several investigators.

Attempts have even been made to find a mathematical formula for this gradual process. As I stated before[1]) MADSEN and NYMAN arrived at the conclusion[2]) that in the disinfection of anthrax spores the reaction proceeds according to the equation for the so-called "unimolecular reactions". This view found favour with most experimenters.

When the reaction is illustrated graphically by plotting the results (abscissae representing the times and ordinates the numbers of survivors), a "curve of survivors" is obtained, having the shape of  . This

[1]) Proceedings of the Meeting of 27 Feb. 1909.

[2]) Z. f. Hyg. u. Inf. Kr. Bnd. 57, 1907.

being an exponential curve, will become a straight line inclining to the abscissae, if we take the logarithms of the numbers of survivors instead of the numbers themselves.

By expressing the results of the experiments logarithmically, we can see at a glance whether, and how far, they are in accordance with the formula, or whether they depart from it; the absolute values being immaterial in this case, I used for my calculations BRIGG's logarithms in place of natural logarithms. (cf. H. CHICK).

In order to account for their results MADSEN and NYMAN regard anthrax spores as an aggregation of individuals of differing resistance. If however this dissimilarity were decisive, a totally different type of "curve of survivors" could be expected, as I demonstrated in the Biochem. Zeitschrift (Bnd, 11. 1908). Conformably to the frequency-curve of QUÉTELET-GALTON an accumulation of deaths could then be expected at an average moment of the process, the rest of the spores with a lower or higher resistance, dying before or after it in gradually lessening numbers. Consequently the curve of survivors would necessarily assume the ⌝-form or, when represented logarithmically, the ⌝-form and not the shape of ⌝. (see also fig. 6, page 637).

Experiments with bacillus coli, published by me in a previous paper really brought forward a curve very much like it, which however differed from the one expected in not being symmetrical, as the first half of the germs were killed in much shorter time than the second.

In the case of anthrax spores I obtained since that time results in fair accordance with MADSEN and NYMAN's experience, just as H. CHICK [1]), REICHENBACH [2]) and others did.

a. Experiments with anthrax spores.

Fig. 1 shows the results of three experiments on disinfection at 80°, 84° and 90°, expressed logarithmically. Their accordance with the formula may be called very satisfactory. The deviations from the straight lines, inclining to the abscissae, are indeed slight. An exception is noticed only at the beginning of the experiment at 80°, where there is hardly a fall in the number of bacteria during the first few minutes. The same had occurred very regularly in my previous experiments with Bacillus coli. This period of lag I then took to be an incubation. I learned since, that an analogous

[1]) The Journal of Hygiene, Vol. VIII 1908, Vol. X, 1910.

[2]) Z. f. Hyg. u. Inf. Kr. Bnd. 60, 1911.

phenomenon is observed in purely chemical reactions, and is called "induction" [1]).

For my experiments I used again suspensions of spores. Of every sample, selected at definite intervals of time, 4—5 parallel cultures were plated, of which I took the average. If the numbers did not mutually agree the experiment was considered to have failed.

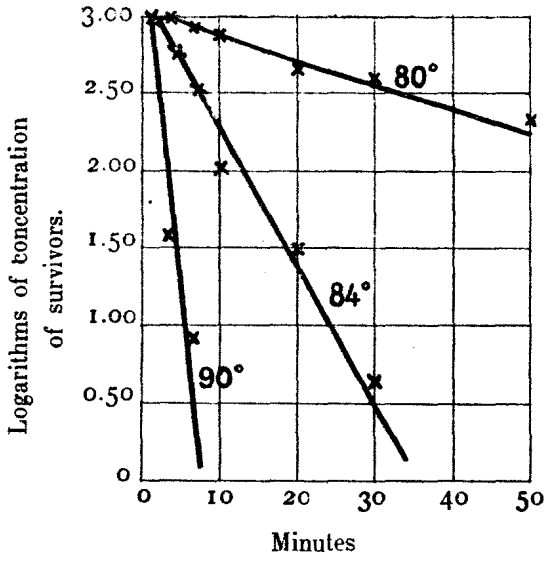

Fig. 1. Disinfection of anthrax spores by heat.

TABLE I. Anthrax spores at 80°.

Time (min.)	Numbers on plates					Mean	Dilution	Number per cc.	Starting number =1000	log_{10}
	1	2	3	4	5					
1	440	422	456	454	454	445	11	4895	1000	3.000
3	431	435	408	454	448	435	11	4785	977	2.990
6	366	343	365	386	406	373	11	4103	838	2.923
10	597	604	605	614	613	607	6	3642	744	2.872
20	724	756	665	729	788	732	3	2196	449	2.652
30	935	950	937	946	921	938	2	1876	383	2.583
50	1159	1081	1077	1022	1024	1073	1	1073	219	2.340

[1]) Bunsen and Roscoe, Pogg. Ann. Bnd. 96, 1855.

For an easy survey and comparison of results I started in my graphical illustration from 1000 living bacteria, the numbers obtained by the experiment underwent a corresponding reduction.

Table I contains the numerical data resulting from an experiment.

As I stated before, MADSEN and NYMAN's interpretation of the conformity in the process of unimolecular reactions and the disinfection of anthrax spores is open to doubt. With greater consistency H. CHICK avers not only that the two processes agree outwardly but are even completely analogous:

"The fact that the individuals do not die all at once but at a "rate proportional to the concentration of the survivors at a given "moment, is to be attributed to temporal and rhythmical changes "in resistance, which by an analogy with chemical processes, may "be supposed to be due to temporary energy changes of the con- "stituent proteins."

Thus putting bacteria on a level with molecules has raised some objections. REICHEL [1]) remarks that this is admissible only if the chances of the germs being attacked by the active mass of the disinfectant were not the same for all bacteria, which in an homogeneous liquid is possible only for particles commensurable as to number and size, such as molecules, not however for micro-organisms and molecules. REICHENBACH thinks so too. He can hardly imagine, that considering the vast difference in size, not all bacteria should be under the same circumstances, relative to the molecules of the germicide. Still less can it be maintained that the bacteria must reach the thermal deathpoint in succession. Moreover considering, that the type of the curve of survivors is not at all determined by the character of the noxious agent, REICHENBACH is induced to think, that the cause is to be looked for only in the micro-organisms themselves, i.e. that differing resistance decides the order of their destruction. The same observer adduces theoretical and experimental evidence to prove, that resistance depends chiefly on the "age" of a generation and shows, by a mathematical treatment, that a culture, having been developed in a definite manner, may contain generations, which, when classified according to their ages form a geometrical series. Assuming moreover that the individual resistance of the cells increases with the age of the generation, this would afford solid ground to account for the orderly progress of disinfection.

It seems to me that this attempt to settle the question is somewhat artificial, its weak point being that REICHENBACH, on the basis

[1]) Biochem. Z. Bnd. 11, 1908.

of his supposition, shows only how a geometrical series *can* come forth, not however why it always *must* do so, for example in the case of anthrax spores, in spite of varying conditions of growth. This points to a regularity as to the age-distribution, which of itself requires an explanation. In my opinion, the one put forward by REICHENBACH is inadequate.

It would seem then that, if we have to find an explanation, the only way would be to consider the progress of disinfection to be mainly a physico-chemical phenomenon. MADSEN and NYMAN and CHICK lend further support to this view by agreeing that VAN 'T HOFF's temperature coefficient appears to be applicable in this case.

It may indeed be called in question, whether this material allows of a mathematical treatment, since it can hardly be worked with without committing serious experimental errors. Consequently, as I pointed out in my first paper, the experimental data of the researchers just mentioned, were far from accurate. Their results however, having been corroborated by several other observers, their opinion that the process of disinfection exhibits some analogy to a unimolecular reaction, can no longer be disputed. Setting aside experimental errors, divergencies from the regular process should then be ascribed to individual differences in resistance.

b. Bacillus coli.

It seems that the individual differences mentioned above are more frequently displayed by vegetative forms than by spores, anyhow they show many more departures from the regular process.

H. CHICK found no less than three types of the curve of survivors for the disinfection of staphylococcus pyogenes aureus with hot water. I also refer to Figs. 2 and 3, giving the logarithmic curves for the disinfection of bac. coli respectively by heat and with 0,5% phenol. It will be seen from Fig. 2 how three coli-cultures *A*, *B* and *C*, though taken from the same stock, when killed by heat, yield very different types. *B* is the only one that corresponds with the type of the unimolecular reaction. *C* shows a marked departure, *A* only a slight one in the opposite direction.

In order to give an idea of the degree of accuracy of this kind of investigations I once more subjoin all the quantitative results of an experiment in Table II. We know that plate-culture is not a very precise quantitative method. Sets of parallel cultures not seldom yield essential differences, even though the sampling may have been

performed with the greatest caution. Our results however, as may

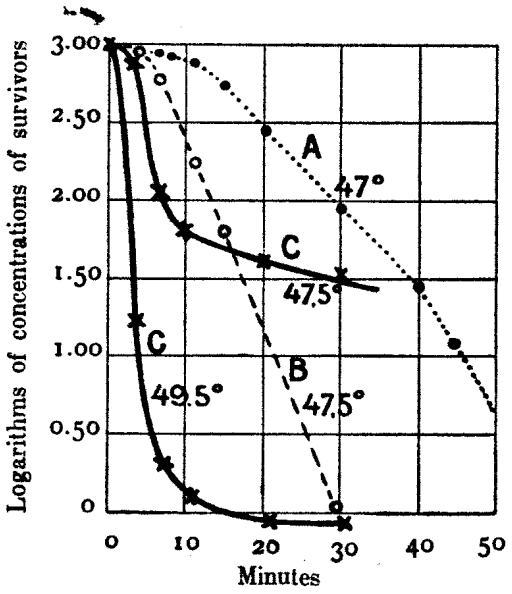

Fig. 2. Disinfection of Bac. coli.
A, *B*, and *C* are different cultures.

be roughly concluded from the table are most likely not more inaccurate than those of other investigators on this subject.

TABLE II. Disinfection of B. coli (culture *C*) by heat at 47.5°.

t (Min.)	Numbers on plates				Mean	Starting number = 1000	log_{10}
	1	2	3	4			
1	2016	2086	2100	2035	2059	1000	3.000
3	1547	1495	1558	1498	1525	740	2.869
6	211	270	221	288	248	120	2.079
10	102	129	137	132	125	61	1.785
20	72	76	65	74	72	35	1.544
30	66	80	65	65	69	33.5	1.525

Disinfection of B. coli with phenol also yielded types of loga-

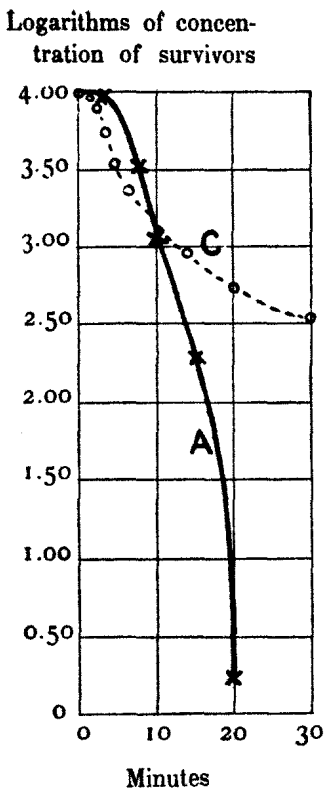

Fig. 3. Colicultur A } 0.5 % phenol at 22°
„ B }

rithmic curves of survivors that differed for various cultures. (Fig. 3.).

Both divert from the straight line, so the reaction- or disinfection velocity is not constant: that of A increases in the progress of the process, whereas that of C diminishes [1]). The same types were also observed by H. CHICK in the case of vegetative organisms.

Type C was also found by REICHENBACH (cf. tab. XIV—XVI l.c.), who worked with very young paratyphus cultures that were killed off by heat at 47—49°. When the culture was older than 13 hours, the exponential curve became smoother, once however it assumed the shape of type A.

REICHENBACH attributes the tendency to depart from the straight line in very young cultures to the relatively large number of low-resistant individuals present. It is remarkable, that H. CHICK's experience is just the reverse: the value of k diminishes in the course of the process for the older cultures, whereas for the younger ones k is smaller and approximately constant.

As for my own experiments (with Bacillus coli), for the sake of uniformity in my material I invariably worked with very young cultures and found, as shown in Figs. 2 and 3 departures in either way. Added to the contradictory results of the observers mentioned above, this seems to suggest that the age of the culture does not determine the form of the curve of survivors.

c. Yeast cells.

It being possible that large cells might lead to other results than small ones, I also made some experiments with yeast cells.

There is perhaps some reason to suppose that speaking generally, in disinfection experiments, whether with thermal or chemical agents, the individuals are destroyed, because the cells, suspended in the liquid, are attacked by molecules, whose caloric velocity exceeds

[1]) The cultures referred to in Fig. 3 and Fig. 2 are not identical, though from the same stock.

a certain limit. A slow process would induce us to think, that these active molecules with a caloric velocity far beyond the average, are only small in number, all the rest being comparatively indifferent. The micro-organisms are then as if were exposed to a continnous shower of bullets (the active molecules) and if this shower be not too dense they will be destroyed in succession and in obedience to the mass-law. Thus the analogy with the unimolecular reaction would be rendered intelligible.

Now, just as in a shower of bullets, the number of "hits" in our case depends on the size of the targets, the larger the individuals are, the more regularly the hits will be distributed among them. We were therefore justified in supposing that, whereas the smaller organisms behave in analogy to the unimolecular reaction, the individual differences of resistance existing among the larger ones become more prominent and express themselves in the form of the curve of survivors.

I do not mean to attach great importance to this illustration, nor to offer its validity as a point to be discussed. I only wanted to set forth why I extended my experiments to larger organisms also.

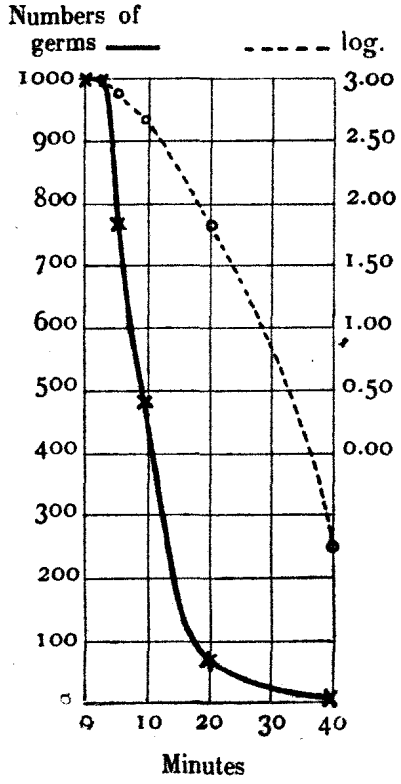

Fig. 4. Rose yeast killed at 47°.

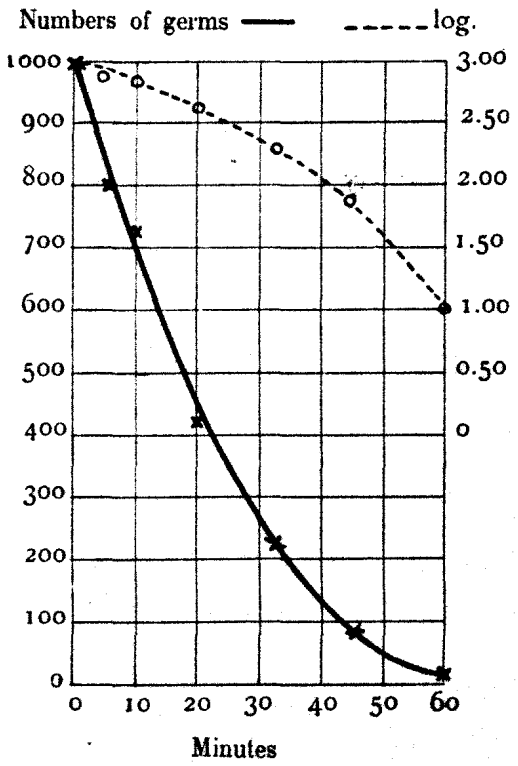

Fig. 5. Rose yeast with 0.6% Phenol at 25°.

First of all experiments were made with *Blastomyces rosea*, a fairly uniform material, consisting of well isolated cells; their size exceeds that of anthrax spores 90 times in volume and twenty times in surface. The curve of survivors corresponds with type *A* of Bacillus coli, i. e. the value of k increases continuously during the experiment (Figs. 4 and 5).

The same type appeared invariably also in working with a pure culture of press-yeast.

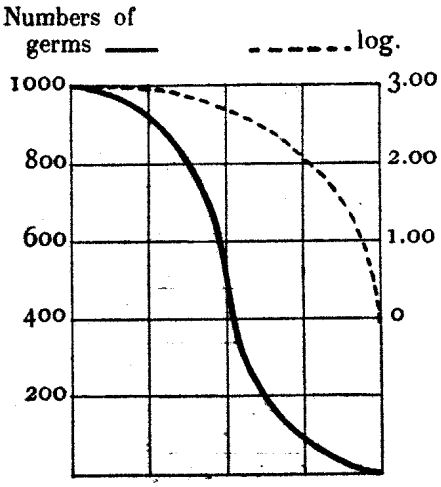

Fig. 6.

Suppose the structure of the cultures according to various degrees of resistance had in this case determined the shape of the curve of survivors, it would not accord entirely with the law of fluctuating variability (QUÉTELET-GALTON). If it did, the curves would look like those in Fig. 6. Intermediate between these and the types of the unimolecular reaction are the curves found for yeast cells.

d. Small and large spores.

REICHENBACH published experiments with the spores of a small saprophytic bacillus. The results differed from those with anthrax spores. The order of dying was not in accordance with the formula for the unimolecular reaction, whether disinfection had taken place by heat or with sublimate. During the process the value of k increased progressively.

As a specimen of small spores I selected those of bacillus subtilis; in all my experiments the results obtained evinced a fair accordance with the formula of the unimolecular reactions. Only towards the end of the reaction k was always inclined to decrease slightly. This peculiarity is indeed also noticeable in my experiments with anthrax spores (cf. Fig. 1). It is much more conspicuous with large spores (see Fig. 8 and Table III). Here we had to do with spores of a particularly big bacillus obtained by chance from the dust settled in a room. Their dimensions are about twice as long as those of anthrax spores. Four experiments, in which the spores were disinfected by

heat showed invariably that there was at a given moment a rather great fall in the disinfection velocity (Fig. 8).

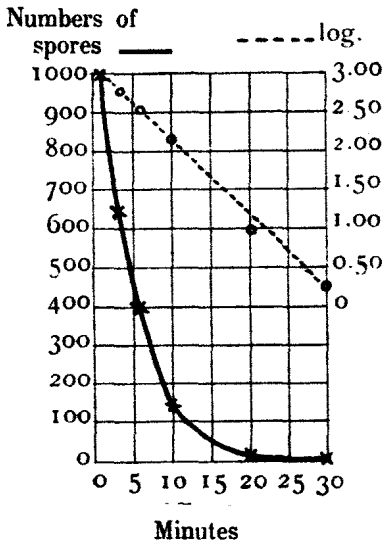

Fig. 7. Small spores at 90°.

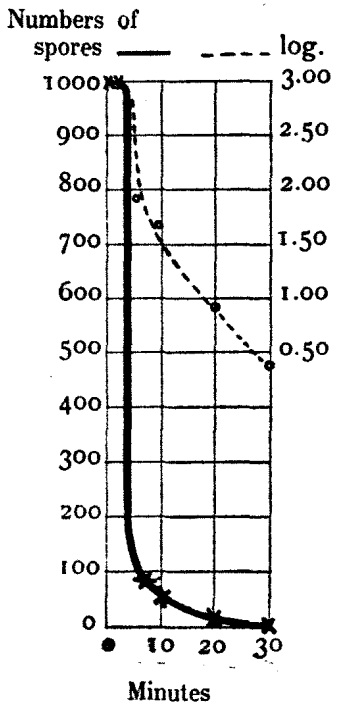

Fig 8. Large spores at 90°.

This result clashes with the reasoning on page 635, which rather implied a gradual rise in the value of k, just as with yeast cells.

TABLE III. Large spores at 90°.

Time (min.)	Numbers on plates					Mean	Dilution	Number per cc.	Starting number =1000	log_{10}
	1	2	3	4	5					
1	940	843	826	830	826	853	75	63975	1000	3.000
3	2610	2624	2600	2603	2571	2602	25	65050		
6	420	—	501	431	487	460	11	5060	78.5	1.895
10	520	481	492	441	481	483	6	2898	45	1.653
20	586	530	593	554	438	540	1	540	8.4	0.924
30	163	151	157	146	112	146	1	146	2.3	0.362

II. *Velocity of germination.*

Germination of spores is to be looked upon as a reaction to the favourable conditions of the nutrient medium. As will appear later on, this reaction can be very rapid at the beginning and is very sensitive either way: in a negative as well as in a positive sense. For when favourable and inhibitory influences coincide, the spores are not to be decoyed from their tents: they do not develop. It seems problable therefore that they permanently keep in touch with their medium, from which they are not isolated by their membrane as completely as is commonly admitted.

According to KOCH and others, who watched the process under the microscope, spores take rather a long time (one or more hours) to germinate. Still in this respect individuals differ greatly. When examining the suspended drop, we shall see after some time besides fully developed spores, others still in their original state, and, between these two extremes, others again in various stages of germination.

We alluded to the possibility of indications of growth being given at the very outset. WEIL [1]), among others, discovered that after 10 minutes' sojourn in broth at 37°, out of 8600 anthrax spores only 60 remained resistant, when heated up to 80° for a short time. This rather surprised him, as he deemed it not likely that the greater portion of the spores should have germinated so rapidly and hence should have become vulnerable at a temperature of 80°. Yet, as also FISCHOEDER [2]) remarks, this is the best way to account for WEIL's experience, which seems to prove that germination can begin very soon, when the circumstances are favourable. Similarly FISCHOEDER found in his microscopic observation of some spores, already after 5—10 minutes, such alterations in their appearance and in their behaviour towards colouring matter as pointed to germination in an initial stage.

The large spores worked with in my experiments on disinfection, published in this paper, were also now selected for my material. Their very size enables us to perfectly control the process of germination. Their growth optimum is 37° C.

The results I obtained, fully confirmed the observations of WEIL and FISCHOEDER. I agree with the latter, that the decrease of resistance towards heat after a short incubation in broth or serum at a favourable

[1]) Arch. f. Hyg. Bnd. 39, 1901.

[2]) C. f. Bakt. I. Bnd. 51, 1909.

temperature, is to be considered as the initial indication of a germinating process, not only on the basis of microscopic observation, but also because of the fact, that there is no decrease, when germination is arrested, for instance by adding to the broth $^1/_2$ % phenol, or by raising its temperature to 50°.

WEIL'S and FISCHOEDER'S numerical data do not practically point to an orderly progress of the germination, which was indeed evidenced by our experiments.

Fig. 9, where logarithms of numbers are plotted against time, illustrates graphically the decrease of the thermostable spores in broth. The logarithmic curves, represented by straight lines, prove that germination proceeds in accordance with the formula for unimolecular reactions.

When germination does not take place at the temperature optimum, in consequence of which the process will be slower, again a period of induction is distinctly noticeable. At 50° there was not any decrease of the resistance, throughout the whole experiment.

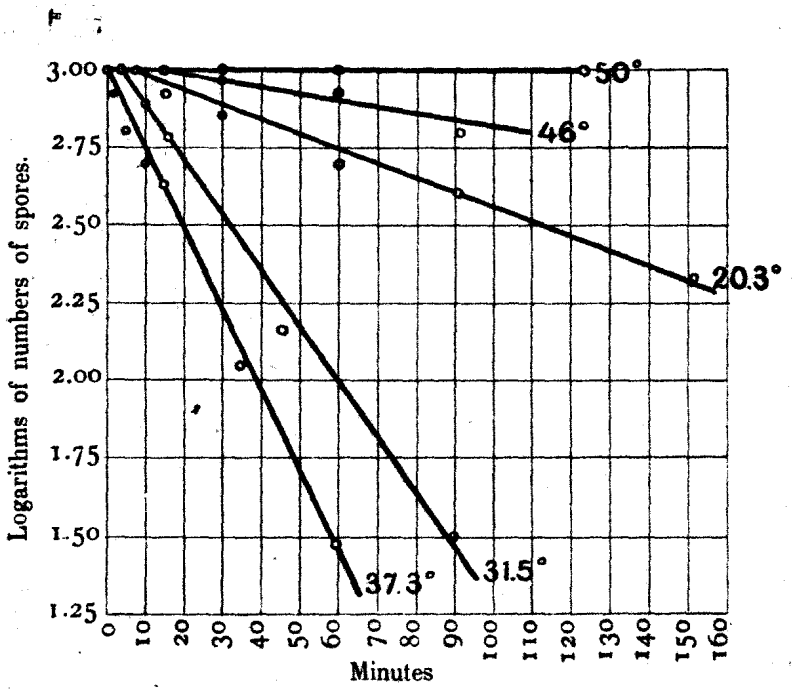

Fig. 9. Large spores in broth at 20.3° 31.5°, 37.3°, 46° and 50°.

The results of one experiment are tabulated in Table IV. Before plating the samples were heated for about five minutes up to 78°.

TABLE IV. Germination of large spores at 31.5°.

Time (min.)	Numbers on plates				Mean	Starting number 1000	log_{10}
	1	2	3	4			
1	489	541	560	534	531	1000	3.000
5	476	583	492	541	523	985	2.993
15	313	340	347	319	330	621	2.793
45	76	90	77	74	79	149	2.173
90	18	16	14	19	17	32	1.505

The results obtained with small spores were entirely analogous with the above. In Fig. 10 the logarithms of the numbers are plotted against time.

On the other hand anthrax spores behave differently as shown in the curves of Fig. 11.

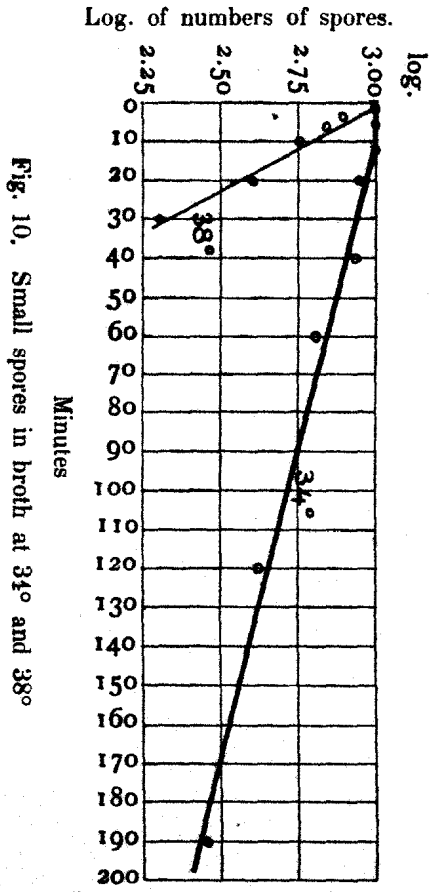

Fig. 10. Small spores in broth at 34° and 38°.

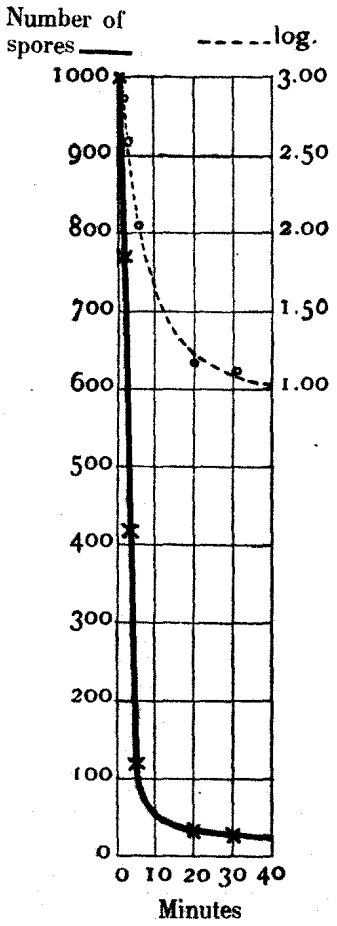

Fig. 11. Anthrax spores in broth at 34°.

The experiments taught that the value of k is not well nigh constant, but diminishes progressively, so that the logarithmic curve is convex on the side of the abscissae. (Fig. 11).

Since it was evident from Fig. 1 that anthrax spores were vulnerable at a temperature of 80°, the samples were heated up before plating to 70° only.

III. *Conclusions.*

1. As regards disinfection of micro-organisms (vegetative forms as well as spores) some species are killed off in an orderly progress analogous to the process of a unimolecular reaction.

In the case of other species the velocity of disinfection is not constant, but either decreases or increases in the course of the process. However with them a certain regularity is also to be observed, viz. apart from the period of induction, the value of k alters in the same experiment continuously in the same sense.

Most often every species has a definite type expressing the orderly progress of its disinfection. Some there are however affording different types in different cultures of the same species; for this variability no satisfactory interpretation can be given.

It is still a matter of doubt, whether the progress of disinfection is chiefly a physico-chemic phenomenon, or whether differing individual resistance of micro-organisms of the same culture play a principal part in the process.

2. A striking analogy is to be observed in the orderly progress

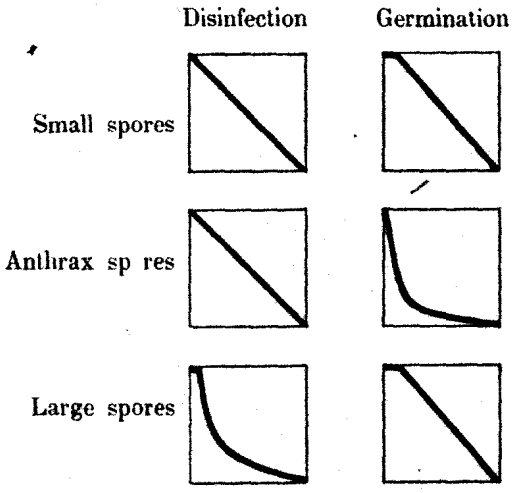

Fig. 12. Types of logarithmic curves.

of germination of spores to that of disinfection. Three species were examined. With two of them development took place in accordance with the formula of the unimolecular reactions.

The reaction-(germinating) velocity of the third species however was not constant, but decreased progressively.

For the same species the orderly progress of disinfection and germination do not always agree as to their types (fig. 12).

Physics. — "*On the second virial coefficient for monatomic gases, and for hydrogen below the* BOYLE-*point*". By W. H. KEESOM. Supplement N°. 26 to the Communications from the Physical Laboratory at Leiden. (Communicated by Prof. H. KAMERLINGH ONNES).

§ 1. *Introduction.* In Suppl. N°. 25 (Sept. '12) a comparison was made between the experimental data at present available concerning the second virial coefficient, B, for monatomic gases, and the relations for the variation of B with temperature deduced in Suppl. N°. 24 (June '12) from certain definite assumptions concerning the structure and the mode of action of the molecules. In continuation of that investigation the present paper supplies a similar comparison for the monatomic gases, and also, in view of the correspondence obtained in § 3*d* of Suppl. No. 25 between these gases and hydrogen below the BOYLE-point, for hydrogen, too, in that region of temperature. Until such time as the theories introduced by NERNST and EINSTEIN concerning the application of the quantum hypothesis to the rotations of the molecules have been further developed, only the suppositions made in Suppl. No. 24*b* § 5 are of any account as simplified assumptions if the specific heats of those gases are taken into account; according to those assumptions the molecules behave as if they were smooth rigid spheres of central structure, attracting one another with a force which is a function of the distance between their centres and is directed along the line joining their centres. As was done towards the end of § 5 of Suppl. No. 24*b*, this function is more closely specified by assuming that the attraction potential may be put equal to $-r^{-q}$ where q is a constant [1]). It

[1]) For the present comparison is postponed with the assumption made by TANNER, Diss. Basel 1912, in which, for simplicity, the action of the attractive force is supposed to be completely localised in a thin concentric spherical shell surrounding the molecule supposed spherical.