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to carbon dioxide. These conditions will doubtless never be fulfilled in land plants, in water plants perhaps very exceptionally.

It has therefore been established by Mr. ZIJLSTRA's investigations, that the speaker was wrong when, in his above cited paper, he came to the conclusion, that a leaf or leaf fragment cannot form starch in a space free from carbon dioxide, when parts organically connected with it, or even immediately adjoining it, are placed in an atmosphere very rich in carbon dioxide. Mr. ZIJLSTRA's results are however, in complete agreement with the main result, formerly obtained by the speaker, according to which the carbon dioxide of the soil, even if it should be absorbed by the roots, cannot appreciably contribute to the synthesis of organic matter in the leaf.

Groningen, January 29th, 1909.

Microbiology. — *"Investigations on the subject of disinfection"*. By Prof. C. EIJKMAN.

Last year I communicated results of experiments [1]) from which it appeared that the resistance against high temperature of bacteria of the same pure culture is individually very different. While for example the majority die off in a few minutes, some may remain alive after $^1/_4$, $^1/_2$ hour, etc. If the times are noted on the absciss and the corresponding numbers of survivors are drawn to it as ordinates, we get as "curve of survivors" a line which in general has the form of a $\curvearrowleft$. In a slow process, as it occurs when the mortal temperature is taken relatively low, the first part of the curve shows itself clearly as an horizontal line and therefore represents a latent stage of incubation. Notwithstanding this the period within which the first half dies off, is much shorter than the following, in which the second half passes away.

In a quick process, as is observed when the temperature is far above the physiological limit, the duration of the incubation will become so brief that it easily escapes notice. In connection with the inevitable circumstance that the number of observations in this kind of experiments cannot be increased arbitrarily, but is confined within a rather definite period, the curve may, instead of the $\curvearrowleft$ form, assume the shape of a $\backslash$.

The latter has also come to light in investigations published the other day by MADSEN & NYMAN, [2]) which differed from mine in so

[1]) Biochem. Zeitschrift, Bnd. XI, Hft. 1—3, Festband Dr. H. J. HAMBURGER gewidmet.

[2]) Z. f. Hyg. u. Inf. Kr. Bnd. LVII.

far as they were not made in vegetative forms of bacteria, but in (anthrax) spores and that the dying off for the greater part was not brought about by heat, but by a chemical means of disinfection, viz. sublimate.

The said investigators think that they are able to give a mathematical formula for their curve. They assert namely that the same formula is applicable here, which holds good for the so-called monomolecular reactions, e. g. for the inversion of cane-sugar by acid:

$$\frac{dx}{dt} = K(a-x).$$

In this formula a represents the number of living anthrax spores that was originally present, x the number that has died off after a space of time t, and K a constant, expressing the velocity of reaction, i. e. the velocity of disinfection. In other words this formula means that during the entire process the number dying off at any moment, is in a constant ratio to the number of living individuals present at that moment.

Therefore this K would yield a very suitable measure to judge about the action of a disinficiens under certain circumstances (of temperature, concentration, etc.). A much better measure than the one customary up till now, viz. the space of time necessary to destroy all germs. For it follows from what precedes that this space of time is to a high degree dependent on the number of germs which in the experiment has been started from. With this number the chance increases that there are some among them which offer resistance extremely long. On the other hand K is not to that degree dependent on the number of germs used in the experiment [1]) and in order to calculate it, the experiment need not even be continued till all germs have died off, but two determinations of x at arbitrary points of time would suffice.

For

$$K = \frac{1}{t_2 - t_1} \ln \frac{A - x_1}{A - x_2}$$ [2])

These experiences of MADSEN and NYMAN have been not only corroborated by an English investigator, Miss HARRIETT CHICK [3]), for anthraxspores and sublimate, but she has stated a similar course of the curve also for the action of three disinfectants on vegetative

[1]) Our experiences render it probable that a great number of germs per unit of volume somewhat retards the process of dying off.

[2]) See the text-books about physical chemistry.

[3]) Journal of Hygiene, 1908.

forms of bacteria, only with this deviation that towards the end the velocity of reaction was decreasing, instead of remaining constant.

On the score of her experiments she is inclined to attribute this deviation to a difference in resistance between the individuals of various ages in the same culture. There would exist, as it were, an old and a young generation by the side of each other, the latter of which dies off slowest. Such a difference in connection with the age does not exist in spores to the same degree.

Of the curves published by me, it is, however, not only the tail, but also the head that shows a deviation. The course is here, still apart from the incubation, much slower than according to the formula. At the most the middle part is, stating roughly, in accordance with it.

Meanwhile it seems to me that this kind of investigations is hardly fit for a mathematical treatment.

MADSEN & NYMAN, for example, avail themselves of means, resulting from numbers of three values found, which deviate 25 % and more from these means.

An example from many [1]):

found:	193,	percentage of the average:	74.5
„	330,	„ „ „ „	127.4
„	254,	„ „ „ „	98.1
average:	259		100

And if the numerical results of Miss CHICK are looked at somewhat more closely, they, too, do not appear to be more exact. Sometimes the errors in the observations are so great that, instead of the expected gradual decrease, here and there an increase of the number of survivors was in course of time to be noted [2]).

It may be called objectionable, as MADSEN & NYMAN do, to rid oneself of the deviations between the numbers determined experimentally and those calculated according to the formula, by remarking: "Wenn man die grossen Versuchsfehler, die an dieser Art von Untersuchungen kleben, in Betracht nimmt, ist die Uebereinstimmung eine recht gute". It is true, a line may be drawn between a number of points determined experimentally, leaving one point to the left, another again to the right, but when, as is not very seldom the case here, the deviations from the regularity are considerable, imagination and arbitrariness will get too large a scope to inspire confidence in the correctness of a curve construed in this way.

[1]) l. c. Table XII.

[2]) l. c. Table III and X.

Seeing that my results did not well agree with those of the above investigators, and this difference might possibly be based upon the fact that the dying off of the microbes was brought about by heat and not by chemical means, I have extended the investigation in this direction. Again bacillus coli communis was made use of, a bacterium forming no spores, while as disinfectant was used phenol in a concentration of at most 1 %, generally only $^1/_2$ %. The use of higher concentrations would make the process of dying off pass so quickly, that the time for a sufficient number of determinations would be too short, and among others the stage of incubation, if at all existing, would easily escape observation.

In order to have no great differences between the individuals and accordingly to render the conditions as little complicated as possible, as a rule a fresh (broth) culture, only a few hours old, incubated at 37°, was taken for the experiments, which culture, in its turn, had also been obtained by inoculation from a fresh culture. For the same reason the broth culture was slowly moved to and fro in a tube specially made for this purpose, which in an apparatus moved by a time-piece had been placed in the thermostate. Consequently all individuals were in well nigh equal conditions of development, so that the results of the experiments were more likely to be equivalent.

Before we used the culture for the experiment, it was centrifugalized in order to remove the clots of bacteria, which were probably to be found in it and for obvious reasons would have a disturbing effect. Besides it was strongly diluted ($\pm$ 1000 times) with physiological common salt-solution. It would be necessary, in order to prevent sowing too many bacteria, to take of the non-diluted broth culture such small samples that, in measuring these, inevitably relatively too great mistakes would be made.

The vessel with the diluting fluid, provided with the necessary quantity of the disinfectant, had already beforehand got the required temperature in a waterbath with a toluolregulator and an automatic stirring-apparatus. After the inoculation with the broth culture the mixture was constantly kept in motion by a glass stirrer, in order to make the disinfectant work as equally as possible upon all germs.

As it is of great importance, to take the samples in rapid succession and just in time, I availed myself for this purpose of a peculiar kind of pipettes, which in case of immersion fill themselves automatically to the required height, so that the measuring, which takes up so much time, was avoided. The samples were put in Petri-scales and sown in melted, lightly alkaline reacting meat-agar. The development took place at 37°. The phenol put in the culture-plate together

with the samples was bound by the alkali and, also because the the quantity was relatively small, it did not disturb the development of the colonies, as appeared from control experiments.

In the graphical representation of the results, to render a mutual comparison easier, a number of 1000 living germs has been started from and the values found experimentally have been reduced accordingly.

As proceeds from the figures, the type of the "curves of survivors" is in our disinfection-experiments with phenol quite similar to the one which was found in dying off by heat; very clearly the ↖ form is again to be recognized in it.

Bac. coli

Fig. 1.

As in our experiments nothing has been left undone to put all the individualls, both in the process of incubation and that of disinfection, under quite the same conditions, a very marked accumulation of deaths might have been expected on either side of an average. In reality, however, this was not the case (most, though, with *a* in fig. 1) and again considerable differences of resistance between individuals of the same culture came to light. For this I see no other explanation, though it remains for the present only a mere supposition, than that the power of resistance during the development between two successive divisions undergoes changes. It may for example be imagined that under for the rest equal circumstances a daughter-cell just formed is, on account of her relatively larger surface, more vulnerable than a full-grown cell.

And because the length of generation is relatively short, amounting, in strong multiplication, to less than half an hour, all stages of development will occur by the side of each other.

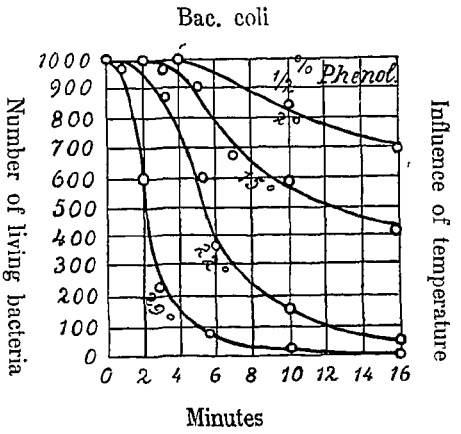

Fig. 2.

As in our former experiments, when the bacteria were killed by heat, we now also experienced, while using phenol as a disinfectant, that, though cultures of one stock are used at every time, yet we did not succeed in coming to somewhat equal results. This is taught by a look at fig. 1, in which a number of eight curves have been drawn, referring to experiments, made at different times each with fresh cultures of the same colistock. The concentration of the phenol and the temperature at which the disinfection took place, were in all cases the same and yet for the greater part quite different curves were obtained.

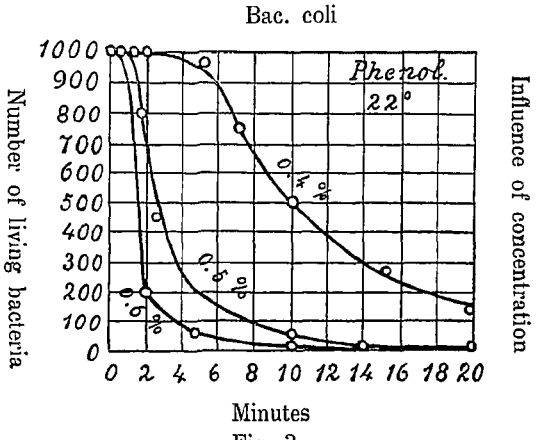

Fig. 3.

Owing to this, it will not do merely to compare the results of one experiment with those of another, unless both are made with the same culture and about the same time. Therefore strictly speaking parallel experiments are necessary, if one wishes to study the influ-

ence of some factor or other, as the concentration, the temperature, on the course of the process.

MADSEN & NYMAN and also Miss CHICK derive from their observations that the influence of the above factors may likewise be expressed in formulas. Thus the well-known formula of ARRHENIUS in which the relation between temperature and velocity of reaction is expressed, would also hold good in this connection. It seems to me, for reasons already mentioned, to be prudent not to follow them on this path. Therefore we refer, with regard to the points meant, to the figures 2 and 3, without commenting on the subject.

Geology. — *"On a long-period Variation in the Height of the Ground-water in the Dunes of Holland."* By Prof. EUG. DUBOIS. (Communicated by Dr. J. P. VAN DER STOK).

Unmistakable and obvious is the lowering that the height of the ground-water in the dunes of the provinces of North- and South-Holland has undergone in consequence of the lowering of the level of the water at their east border (the making dry of the Lake of Harlem and of a large part of the IJ) and of deep cuttings in the dunes themselves (North-Sea Canal), furtheron, not less, by the collecting of large quantities of water supplies for some cities and towns.

From these causes there resulted a lowering which may be called a permanent one, inasmuch that soon they have brought about a new state of equilibrium with the supply by the part of rainfall which soaks in, and the flowing off. This really did take place in each case in which certain limits were not transgressed and as long as the collecting of water did not increase.

Side by side with these artificial changes of the height of the ground-water in the dunes, there exist also changes by natural, viz. climatal causes. These, in this as in other cases, are not continuous, but they do occur in periods. Indeed, in the latest historical past, as far as data are available, very clearly dry and wet epochs alternate with one another.

The Commission which, in 1891, inquired in the supplying of water from the dunes to Amsterdam pointed out, in their report, that from 1849 till 1856 there was a period of much rain, from 1856 till 1868 a dry period, again followed by the rainy years of 1869 till 1882. They showed also (for Utrecht) that under the combined influence of rainfall and evaporation such wet and dry epochs are