MULTIPLICATION OF BACTERIA AND THE INFLUENCE OF TEMPERATURE AND SOME OTHER CONDITIONS THEREON.

By JANET E. LANE-CLAYPON, M.B., D.Sc.

Jenner Memorial Scholar, Lister Institute of Preventive Medicine.

1 Figure.

THE methods which have been employed for estimating the rate of growth of bacteria are numerous.

Nägeli (1887) endeavoured to estimate the rate of growth by studying the amount of acid produced by an acid-producing organism.

Buchner, Longard and Riedlin (1887) studied the rate of growth of the cholera bacillus, using the plating-out method of Koch. They plated-out the culture at the beginning and at the end of a period of from 2—5 hours. The generation time was calculated from the formulae

$$2^n = \frac{b}{a}$$ where a = No. of bacteria at beginning,

b = No. of bacteria at end

and n = No. of generations,

and $$G = \frac{T}{n}$$ where G = generation time and T = time of experiment.

The results obtained for G varied from 19 to 40 minutes at 37° C. This appears to be a large difference, but, as pointed out by Hehewerth (1901), Buchner did not allow for the initial "lag" which most observers find when a fresh culture is made.

Rahn (1900), working chiefly with *B. fluorescens*, has studied the question of this early lag; he found that the minimum generation time at 24° C. occurred 6—21 hours after inoculation. After this the growth was very slow up to about 48 hours.

Klein (1900) counted the number of stained bacteria in a given field, knowing the area of the field in its relation to the area covered by a drop spread out on a film.

Hehewerth (1901) published a number of experiments on the growth of *B. coli* and *B. typhosus*. He worked with large numbers of bacteria, the first estimation generally giving a count of several millions.

https://doi.org/10.1017/S0022172400016260 Published online by Cambridge University Press

He found that there is an initial period after inoculation during which growth is almost absent: the length of time of this "lag" varies with the age of the culture used for inoculation and with the species of the bacillus. With a 19 hours old culture of *B. coli*, the lag at 37° C. lasted rather over one hour: with *B. typhosus* it lasted over two hours.

A culture having once started growing, the numbers run up quickly and then remain fairly constant for some time, subsequently slowly decreasing.

The generation time of *B. coli* at 37° C. in broth he found to be 21—31 minutes, the average being 23 min. 24 sec. and in peptone water to be 28—32 minutes, or sometimes rather more, depending upon the age of the culture. The generation time for *B. typhosus* averaged 33 min. 24 sec. in broth, and 45 min. 37 sec. in peptone water.

Boland (1902) tried to estimate rate of growth by using a standard turbidity.

Müller (1903) used cultures of bacteria isolated by him from frozen material: with slight variations the generation times were the same for the different bacteria at the same temperature, viz., about 50—60 minutes at 30° C. during the first 12 hours, with a lag of nearly two hours; and about 54—70 minutes at 25° C. with a lag of about three hours, or rather more.

He usually started the experiments with a culture containing about 1000—2000 bacteria per c.c.

Barber (1908) worked chiefly with *B. coli*. By means of a fine capillary pipette he removed a single bacterium and determined the actual rate of division. He found no preliminary lag if the bacteria were inoculated into a medium to which they were accustomed. He found that the generation time gradually decreased up to about 40° C., after which it increased.

For *B. coli* he obtained the following times with individual variations of several minutes:

20° C. ..... 60 mins.	40° C. ..... 17 mins. or rather more.
25° C. ..... 41 mins.	42° C. ..... 19—20 mins.
30° C. ..... 29·7 mins.	45° C. ..... 30—34 mins.
37° C. ..... 17—21 mins.	50° C. ..... no growth.

His figure for 37° C. is lower than that of other observers.

The rate of multiplication of bacteria increases from 2—3 times between the temperatures of 20° C. and 30° C. (cp. Hehewerth and Barber).

The optimum temperature differs for different species, and the temperature at which they are kept as stock cultures in the laboratory has been found to have some influence in determining the subsequent rate of growth at any particular temperature.

Methods.

Throughout these experiments the same method has been used, the details being reproduced each time as far as possible.

The cultures used had all been kept at room temperature for over one year. The species of bacteria used were *B. coli, B. typhosus* and *B. enteritidis* Gaertner. A fresh agar culture was made and kept

at room temperature for 24 hours and a sub-culture was made from this into about 5 c.c. of broth medium.

The broth culture was then allowed to grow for about 20 hours also at room temperature. With the organisms employed, it was found by experiment that a millionfold dilution of this culture afforded a suitable number of organisms to use for the beginning of an experiment, viz., 200—500 bacteria per c.c.[1]

The culture was kept in the dark at the desired temperature. The temperature varied within half a degree centigrade of that recorded.

At intervals after incubation a definite number of drops (1 drop = ·02 c.c.) were removed by means of a standard capillary pipette and plated.

The actual number of drops required to produce a reliable plate at different periods of incubation had to be discovered by experiment. Two or three plates were made for each estimation.

In the later stages of the experiments the culture required dilution before plating; this was carried out as rapidly as possible by dropping one drop of the culture into the required amount of sterilised water, shaking well, and then with another similar pipette the desired number of drops of the dilution were plated as usual. The whole time required was about two minutes, and control experiments showed that there was no deleterious effect from the distilled water during this short time.

Results of Experiments.

The observations show that there are four phases in the bacterial life of a culture:—(1) an initial period of slow or of no growth; (2) a period of regular growth, the rapidity varying slightly at the same temperature, but differing widely for different temperatures; (3) a period when the numbers remain more or less stationary; (4) a period when the numbers of living bacteria are diminishing.

Period I.

All observers, except Barber (1908), record an initial lag varying in extent at different temperatures. In all my experiments I found a very definite lag, during which the number of bacteria per drop remained almost constant.

[1] The number of organisms mentioned throughout this paper refers to estimates formed from plating out on agar. This method does not give 100 % of the organisms present but with the same sample of culture medium affords a constant error.

With *B. coli* and *B. enteritidis* Gaertner this latent period was found to extend to from one to six hours as the temperature varied from 42° C. to 20° C. In the case of *B. typhosus* the lag was rather longer at each temperature.

This lag agrees almost exactly with that observed by Hehewerth (1901) who used cultures of very nearly the same age (19 hours). Rahn (1906) states that it is less if the inoculation is fairly heavy.

Period II.

The lag being over the bacteria now enter upon a phase of rapid growth.

The rate of growth has been calculated by various observers on the assumption that the bacteria are all in a state of active and regular division so that the number increases logarithmically. By the method described, I have been able to ascertain that this is the case for a considerable length of time after the culture first starts growing.

The actual figures showing the increase in numbers of *B. coli*, *B. typhosus* and *B. enteritidis* Gaertner, at temperatures 20—42° C. are set out in Tables I, II and III respectively. In parallel columns are placed the average logarithmic differences per hour, the proportional increase in number per hour and the mean generation times as calculated[1] from the observations.

In Fig. 1 the logarithms of the numbers of *B. coli* found in unit volume at different intervals have been plotted against time. This has been done for various temperatures and the points fall upon straight lines. The slope of these lines is different at the various temperatures, the rate of growth being proportional to the tangent of the angles made by them with the abscissa.

Similar graphs are obtained if the figures for *B. typhosus* and *B. enteritidis* Gaertner be also plotted in the same way.

[1] During the time that growth proceeds logarithmically the number of generations in any interval of time $t_1 - t$ is measured by the power to which 2 must be raised to produce the same increase as the proportion between the number of bacteria at time t_1 and t.

$$2^n = \frac{\text{number at } t_1}{\text{number at } t};$$

taking logarithms the equation can be expressed:

$$n = \frac{\log \text{ number } t_1 - \log \text{ number } t}{\log 2};$$

n is the number of generations in time $t_1 - t$ and the generation time is $\frac{t_1 - t}{n}$.

https://doi.org/10.1017/S0022172400016260 Published online by Cambridge University Press

In the experiments in the tables, the ascertained period of "lag" was allowed to elapse before the first observation was made.

For a given volume of culture fluid the time during which the bacteria continue to divide at a maximum rate depended upon the insemination and the temperature, being shorter if the inoculations were heavy and at the higher temperatures.

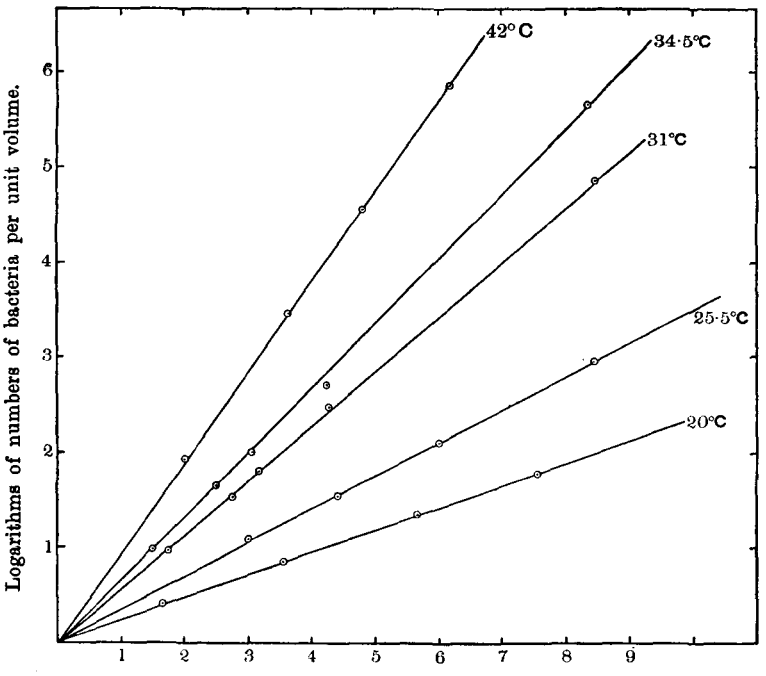

Fig. 1.

The results recorded below are those obtained with an insemination of 200—500 per c.c. Under these circumstances, and discounting the period of lag, the length of time over which *B. coli* and *B. enteritidis* Gaertner maintained the maximum rate was approximately:

$7\frac{1}{2}$	hours	at	37° C.
8—$8\frac{1}{2}$	„	„	30° C.
12—15	„	„	25° C.
20—24	„	„	20° C.

B. typhosus grew more slowly and the logarithmic increase persisted longer at all temperatures.

https://doi.org/10.1017/S0022172400016260 Published online by Cambridge University Press

At 20° and 25° C. the logarithmic increase was maintained almost up to the time when the medium contained a maximum number of living bacilli, but at the higher temperatures the rate fell off considerably before this point was reached.

Periods III and IV.

After the culture has ceased growing logarithmically the rate of growth slackens gradually, but is still fairly active until the number of bacteria reaches several million per drop (·02 c.c.), that is several hundred million per c.c. At this stage the number of living bacteria present appears to remain fairly constant for some time (from two to five days according to the temperature) after which it begins to decrease slowly.

The results obtained with *B. coli* resembled those of *B. enteritidis* Gaertner, except that the maximum number obtained is about double.

There appears therefore to be a maximum number of bacteria which a unit volume of medium is capable of supporting: this means that for that particular organism the conditions are no longer favourable for increased growth. This may be due to the using up of some constituents of the broth or it may be due to some inhibitory substance produced by the organisms themselves in the process of metabolism. I have not carried out any investigations on these points. 400,000,000 to 800,000,000 per c.c. of broth seems to be the maximum for *B. coli* and *B. enteritidis* Gaertner.

After two days at 37° C. and sometimes before, it is difficult to get an accurate estimation as there is a great tendency to agglutinate, especially with *B. coli* and *B. enteritidis* Gaertner.

Influence of temperature upon generation time.

The effect of temperature was studied over the range between 20 and 50° C. Up to 42° the generation time was reduced, but above this it apparently increased, and at 50° diminution replaced increase in the number of organisms.

The experiments of Hehewerth (1901) and Barber (1908) showed that the generation time was reduced one-half to one-third by raising the temperature from 20° to 30° C. This is an effect of the same order as that of temperature upon many common chemical reactions. The logarithms of Barber's values for mean generation times between 20° and 37° C. plotted against temperature fall upon a straight line,

showing that each rise of one degree throughout this range produces the same proportionate effect.

My observations for *B. coli* and *B. typhosus* are in accord with those of Barber. The influence of temperature upon the growth of *B. enteritidis* Gaertner I find to be decidedly less. The temperature effect upon the growth of these three organisms is fairly constant between 20° and 35° C. Each rise of one degree produces the same proportionate increase in the rate of growth. The logarithms of the mean generation times (20°—35°) from Tables I, II and III plotted against temperature show a linear relationship, signifying that the effect of temperature upon the growth of these bacteria accords with the Arrhenius-Van 't Hoff law within the error of the experiments.

The increase in rate of growth per 10° rise in temperature obtained from the drawn lines is 2·2 for *B. coli* and *B. typhosus* and 1·7 for *B. enteritidis* Gaertner.

Above 35° C. the effect of temperature in diminishing the mean generation time is, as found by Barber, distinctly less.

Summary.

The species of organisms used were *B. coli*, *B. typhosus* and *B. enteritidis* Gaertner. With these organisms:

(1) When a fresh broth culture is made with a small inoculation there is a period during which there is no increase in the number of bacteria present.

(2) When this period is over the bacteria commence to divide regularly; this is shown by the fact that the logarithms of the numbers plotted against time are found to fall on a straight line. This regular growth persists until (or nearly until) a maximum has been reached, after which the numbers remain more or less constant and then slowly decline.

The time necessary for a complete division to take place (generation time) was determined for various temperatures between 20° C. and 42° C.

(3) The effect of temperatures between 20° and 35·3° C. upon the rate of multiplication is in accordance with the Arrhenius-Van 't Hoff law; above this temperature the effect diminishes.

I have much pleasure in thanking Dr C. J. Martin for his invaluable assistance and interest throughout this research and also Miss Müllenbach for kindly preparing the diagram.

TABLE I.

Growth of B. coli at temperatures 20° *to* 42° C.

Temp.	Time after commencement of experiment hrs.	mins.	No. of bacteria per drop	Logarithm of number per drop	Average Log. difference per hour = velocity constant	Proportional increase in numbers per hour	Mean generation time in hours
42° C.	2	0	87	1·94	·94	8·7	·32
	3	40	2,876	3·46			
	4	50	36,675	4·56			
	6	10	739,200	5·87			
34·3° C.	1	30	9	·97	·68	4·8	·44
	2	30	43	1·63			
	3	5	105	2·02			
	4	15	499	2·70			
	8	22	476,666	5·68			
31° C.	1	45	10	1·00	·57	3·7	·53
	2	45	34	1·53			
	3	10	65	1·81			
	4	22	288	2·46			
	8	27	72,533	4·86			
25·5° C.	3	0	12	1·08	·35	2·2	·86
	4	25	33	1·52			
	6	0	128	2·11			
	8	27	893	2·95			
20° C.	12	15	243	2·38	·23	1·7	1·30
	14	10	720	2·85			
	16	15	2,166	3·33			
	18	10	5,066	3·70			
	22	30	46,666	4·67			

TABLE II.

Growth of B. typhosus at temperatures 20°—34·3° C.

34·3° C.	1	30	7	·84	·55	3·5	·55
	2	30	24	1·38			
	4	0	152	2·18			
	9	20	195,000	5·29			
	26	20	4,133,000	6·62			
31° C.	1	50	7	·84	·40	2·5	·75
	2	45	17	1·23			
	4	30	82	1·91			
	9	25	13,600	4·13			
	26	25	3,966,000	6·98			
25° C.	3	50	10	1·0	·23	1·7	1·30
	6	10	36	1·56			
	9	35	178	2·25			
	26	30	3.566,000	6·55			
20° C.	6	15	10	1·0	·18	1·5	1·67
	8	37	28	1·45			
	9	38	36	1·56			
	26	35	51,145	4·71			
	54	10	4,933,000	6·69			

TABLE III.

Growth of B. enteritidis Gaertner at temperatures 20° *to* 42° C.

Temp.	Time after commencement of experiment		No. of bacteria per drop	Logarithm of number per drop	Average Log. difference per hour = velocity constant	Proportional increase in numbers per hour	Mean generation time in hours
	hrs.	mins.					
42° C.	2	20	83	1·92			
	4	0	1,940	3·28	·81	6·5	·37
	5	0	13,417	4·12			
	6	20	176,400	5·24			
34·3° C.	1	30	13	1·12			
	2	30	43	1·63			
	3	0	80	1·90	·59	3·9	·51
	3	30	179	2·25			
	6	15	8,960	3·95			
	12	25	3,580,000	6·55			
	25	30	5,366,000	6·72			
	49	40	10,833,000	7·03			
	121	0	6,266,000	6·79			
31° C.	1	45	13	1·11			
	2	45	44	1·64			
	3	15	85	1·92	·54	3·5	·56
	6	35	4,050	3·60			
	8	15	44,000	4·64			
	12	30	1,480,000	6·17			
	25	30	9,000,000	6·95			
	49	40	8,400,000	6·92			
	121	0	4,600,000	6·66			
26° C.	4	0	25	1·40			
	4	45	57	1·75			
	5	30	112	2·04			
	6	15	208	2·31	·42	2·6	·71
	6	45	361	2·55			
	12	0	56,933	4·75			
	25	30	9,125,000	6·96			
	49	40	9,600,000	6·98			
	121	0	10,466,000	7·01			
20° C.	6	40	28	1·44			
	7	45	56	1·75			
	8	35	98	1·99	·29	2·0	1·0
	12	45	1,546	3·18			
	26	0	7,581,400	6·87			
	49	40	10,433,000	7·01			
	121	0	10,066,000	7·00			

https://doi.org/10.1017/S0022172400016260 Published online by Cambridge University Press

BIBLIOGRAPHY.

BARBER (1908). The rate of multiplication of *B. coli* at different temperatures. *Journ. of Infectious Dis.* v. 379.

BOLAND (1902). Inaug. Dissert. Amsterdam.

BUCHNER, LONGARD u. RIEDLIN (1887). Über die Vermehrungsgeschwindigkeit der Bakterien. *Centralbl. f. Bakt.* I. Abt. Vol. II. p. 1.

HEHEWERTH (1901). Die mikroskopische Zählungsmethode der Bakterien von Alex. Klein, und einige Anwendungen derselben. *Arch. f. Hyg.* XXXIX. 321.

KLEIN (1906). Eine neue mikroskopische Zählungsmethode der Bakterien. *Centralbl. f. Bakt.* I. Abt. Vol. XXVII. p. 834.

MORGAN (1906). Upon the Bacteriology of the Summer Diarrhoea of Infants. *Brit. Med. Journ.* I. 908.

MÜLLER (1903). Über das Wachstum und die Lebensfähigkeit der Bakterien, sowie den Ablauf fermentativer Processe bei niederer Temperatur, unter spezieller Berücksichtigung des Fleisches als Nahrungsmittel. *Arch. f. Hygiene*, XXXVII. 127.

NÄGELI (1877). *Das Mikroskop.*, 2nd Aufl. Leipzig, p. 461.

RAHN (1900). Über den Einfluss der Stoffwechselprodukte auf das Wachstum der Bacterien *Centralbl. f. Bakt.* II. Abt. Vol. XVI. p. 417.