THE GROWTH RATE OF INDIVIDUAL BACTERIAL CELLS

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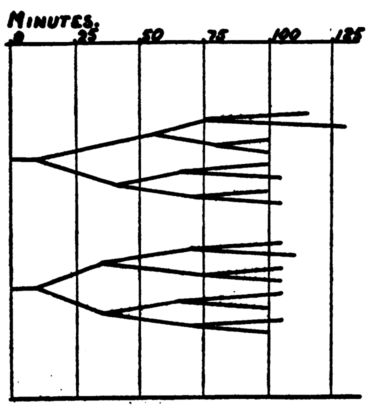
It has been assumed by many bacteriologists that during the period of rapid growth, in a satisfactory culture medium, some bacteria will die in spite of good food and favorable environment. No doubt this assumption was derived from an analogy with populations of higher forms of life, of which a number of individuals are known to die before they reach the reproductive age even with good care.

Wilson (1922) approached this question experimentally, and found that there was a regular discrepancy between plate counts and direct microscopic counts which he explained by supposing that some bacteria would not grow on agar but could still be seen. Reichenbach (1911), in order to explain the logarithmic order of death, assumed that a certain proportion of the multiplying bacteria of each new generation ceased multiplying and became dormant, and that the resistance of these dormant forms increased with age; thus a logarithmic gradation of resistance might be established.

These assumptions have been tested by us in the following manner: Bacteria, and in later experiments yeasts, from young liquid cultures were spread on the surface of an agar plate; from this seeded plate, a square was cut out and used as a hanging block in a moist chamber, for direct observation of multiplication under the microscope. The technique observed was essentially that of Orskow (1922), but the same field was kept under the microscope during the entire observation, which usually covered four generations. The moist chamber was held in a stage incubator where the temperature was maintained at 30°C., for all experiments.

A field was located in which there were 7 or 8 isolated cells and every five minutes a graphic sketch was made of all cell divisions,



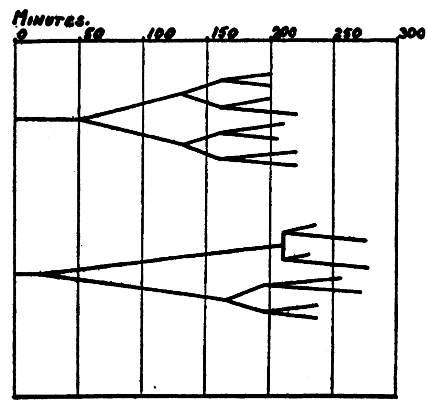


FIG. 1. *Bacterium aerogenes* FIG. 2. *Bacillus cereus*

Chronological record of the growth of bacteria from one cell, where each branching indicates the time when a cell division occurred.

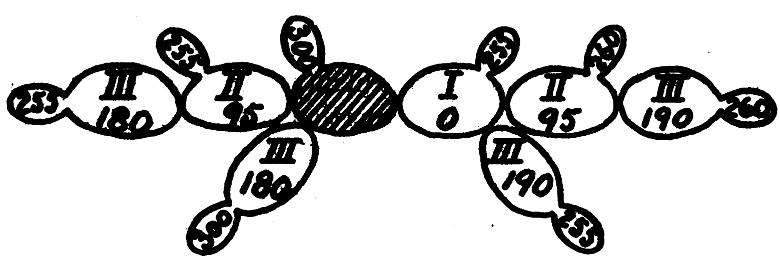


FIG. 3. YOUNG COLONY OF *Saccharomyces ellipsoideus*

The shaded cell is the mother cell. Roman numerals indicate successive generations, Arabic numerals mean "birth dates," i.e., the number of minutes when the cell appeared as a bud, taking the appearance of the first generation (cell I) as zero time.

similar to that shown in figure 3. In this manner the "family tree" was kept to the fourth generation, each original cell increasing from one to sixteen. Figures 1, 2 and 3 depict graphically the fission or budding times of the progeny of five typical cells.

Longer observation was impractical as it was not possible after this to tell from which mother cell the newly divided cells had come.

TABLE 1

Frequency of fission times of Bacterium aerogenes at 30°C., covering three generations

GENERATION	GENERATION TIME							INDIVIDUAL CELLS
	10–15 minutes	15–20 minutes	20–25 minutes	25–30 minutes	30–35 minutes	35–40 minutes	40–45 minutes	
II		3	8	4			1	16
III	2	3	13	12	2			32
IV	4	17	22	14	7			64

TABLE 2

Frequency of fission times of Bacterium aerogenes

	FISSION TIME														TOTAL
	5–10 minutes	10–15 minutes	15–20 minutes	20–25 minutes	25–30 minutes	30–35 minutes	35–40 minutes	40–45 minutes	45–50 minutes	50–55 minutes	55–60 minutes	60–65 minutes	65–70 minutes	70–75 minutes	
February 17..........					2	13	12	7	7	2	1				44
February 24..........			2	3	4	20	16	11	2	1		1			60
March 3.............		1	3	12	20	38	19	18	8	4	1	1		1	126
March 10............			6	10	16	26	18	9	3	1				4	93
Total..............		1	11	25	42	97	65	45	20	8	2	2		5	323
March 2.............	1		3	19	22	21	10	5	2	1					84
March 6.............			4	12	30	19	15	1		2	1				84
Total..............	1		7	31	52	40	25	6	2	3	1				168
March 17............		6	23	43	30	9		1							112
November 12.........		·	13	60	24	3									100
November 14.........			5	13	4	5	2	1							30
Total..............		6	41	116	58	17	2	2							242

Table 1 gives the detailed record of an experiment with *Bacterium aerogenes*. Eight cells were located in one field and these were found to divide between five, and twenty minutes after observation had started. This table shows between which of the five-minute periods the second, third and fourth divisions took place. In this way many observations were made, summaries of which are given in tables 2, 3 and 4. In some cases, especially with *Bacterium aerogenes*, a lag was to be noted in the first recorded generation, and where this has occurred the data, for this generation, have not been recorded in tables 2, 3 and 4. It hap-

TABLE 3

Frequency of fission times of Bacillus cereus

	FISSION TIME																												
	0–5 minutes	5–10 minutes	10–15 minutes	15–20 minutes	20–25 minutes	25–30 minutes	30–35 minutes	35–40 minutes	40–45 minutes	45–50 minutes	50–55 minutes	55–60 minutes	60–65 minutes	65–70 minutes	70–75 minutes	75–80 minutes	80–85 minutes	85–90 minutes	90–95 minutes	95–100 minutes	100–105 minutes	105–110 minutes	110–115 minutes	115–120 minutes	120–125 minutes	125–130 minutes	130–135 minutes	135–140 minutes	TOTAL
March 24	3		1		6	2	8	7	9	18	16	5	6	3	5		1	2		1	1		1	1					96
March 30	2	2	2	4	5	8	8	11	5	6	3	1	6	2	2	2	1		2				1	1		1	1		76
April 1	4		1	1	6	5	4	5	4	10	6	6	9	3	5	3	5	1	2	1	2	1							84

TABLE 4

Frequency of budding times of Saccharomyces ellipsoideus

	FISSION TIME																											
	45–50 minutes	50–55 minutes	55–60 minutes	60–65 minutes	65–70 minutes	70–75 minutes	75–80 minutes	80–85 minutes	85–90 minutes	90–95 minutes	95–100 minutes	100–105 minutes	105–110 minutes	110–115 minutes	115–120 minutes	120–125 minutes	125–130 minutes	130–135 minutes	135–140 minutes	140–145 minutes	145–150 minutes	150–155 minutes	155–160 minutes	160–165 minutes	165–170 minutes	170–175 minutes	175–180 minutes	TOTAL
April 21	6			2		6	14	44	49	29	21	19	4	2	2	5	9	6	6									224
April 28												4	22	16	32	38	32	21	17	2	6		2	2				196

pened in 3 cases out of 110 that a cell did not divide in the new medium. An explanation for this might be found in the paper of K. A. Jensen (1928). But, if a cell divided once, all of its offspring continued to divide. There was not a single instance observed where a cell ceased multiplying, became dormant or died.

If the rate of death during "infancy" were the same for bacteria and yeasts as for the higher animals and plants, it should be possible to detect this by the method used providing a sufficient number of individual cells had been observed. Altogether 1,766 cells were kept under observation: *Bacterium aerogenes*, 977 individual cells; *Bacillus cereus*, 325 individual cells; *Saccharomyces ellipsoideus*, 464 individual cells.

It must be remembered, however, that this investigation covers only the first 4 generations after transfer to a new medium.

VARIATIONS IN THE RATE OF GROWTH

It is to be noted in tables 2 and 3 as well as on figures 1 and 2 that in the present investigation, considerable variation was observed in the rate of growth of individual cells under uniform conditions. This variation was shown not to be inheritable except for one yeast cell. In this case, six cells (table 4), all the progeny of one parent cell, were found to multiply in from forty-five to fifty minutes and 2 others from the same cell in sixty to sixty-five minutes, while the average was eighty-five to ninety minutes. This was the only example where fast growth appeared to be inherited. As a general rule, however, we do not have a mixture of fast-growing and slow-growing strains, but the two cells arising from the division of one cell may have greatly differing generation times. In table 5, the progeny of the fastest and the slowest growing cells of *Bacterium aerogenes* are shown to divide in a normal manner and are not greatly influenced by the dividing time of the parent cell. Since the progeny of the fastest cells multiplies not faster than the average, while that of the slowest cells is a little faster, it seems that the division of cell walls may be delayed after the cell contents have divided.

This is a common occurrence with spore-formers. A cell grows in length for a considerable time without dividing, and then when it finally divides, the next division comes very rapidly. This is to be seen in the second figure of figure 2, where one cell does not divide for one hundred eighty-five minutes, while the following division comes in twenty-five minutes. A more exaggerated example is to be found in the other half of the same figure, where the second and third divisions come at the same time interval.

In one case, with *Bacterium aerogenes*, the cell elongated without showing a division, and though the cell appeared as one, it continued to function as two cells. The next division occurred at the normal time. Each half of the cell divided as if it were an individual cell, breaking off a quarter of the entire length for the new cell. In the next generation the cell behaved again in the

TABLE 5

Fission times of the progeny of the fastest and slowest growing forms of Bacterium aerogenes

	FISSION TIME												
	5–10 minutes	10–15 minutes	15–20 minutes	20–25 minutes	25–30 minutes	30–35 minutes	35–40 minutes	40–45 minutes	45–50 minutes	50–55 minutes	55–60 minutes	60–65 minutes	65–70+ minutes
Progeny of the fastest growing cells													
February 17	—	—	—	—	—	—	1	—	1	—	—	—	—
February 23	—	—	—	—	—	2	—	4	1	—	1	—	—
February 24	—	—	—	—	—	9	3	2	—	—	—	—	—
March 2	—	—	—	12	2	—	—	1	1	—	—	—	—
March 3	—	—	—	1	3	7	2	2	—	1	—	—	—
March 6	—	—	2	—	3	8	4	—	—	1	—	—	—
March 10	—	—	—	—	4	9	—	1	1	—	—	—	3
March 17	—	3	5	5	2	1	—	—	—	—	—	—	—
Total	—	3	7	18	14	36	10	10	4	2	1	—	3
Progeny of the slowest growing cells													
February 17	—	—	—	—	—	4	1	1	2	2	—	—	—
February 23	—	—	—	—	—	—	2	—	7	—	—	—	1
February 24	—	—	1	1	—	—	—	—	—	—	—	—	—
March 2	1	—	1	1	—	—	1	1	—	1	—	—	—
March 3	—	1	2	3	—	—	—	—	—	—	—	—	—
March 6	—	—	1	2	1	—	—	—	—	—	—	—	—
March 10	—	—	2	3	1	—	1	—	—	—	—	—	—
March 17	—	1	2	1	—	—	—	—	—	—	—	—	—
Total	1	2	9	11	2	4	5	2	9	3			1

same way, while the offspring doubled normally. This cell might be compared to the "Siamese Twins."

A chemical explanation of the cause of variation in growth rates of unicellular and multicellular organisms will be given elsewhere by one of the authors (Rahn, 1932).

SUMMARY

1. Microscopic observation of more than 1,700 individual cells of bacteria and yeasts showed that under favorable conditions of growth, all cells continue to multiply if they once have started to do so, and there was not a single case of "infant mortality" observed. The observations extended over the first 4 generations after transfer to agar.

2. There is considerable variation in the rate of fission (or budding) of the individual cells. This is not an inherent property of certain strains in the culture because the offspring of the fastest and slowest cells is not fast nor slow, but average.

The division of the cell contents (mitosis) is not always followed promptly by a division of the cell into two, and it may happen with spore-formers that one cell divides into 4 cells during the stage of fastest growth.

REFERENCES

Jensen, K. A. 1928 Centralblatt für Bakteriologie, 1 Abt., Orig., **107**, 1.
Orskov, F. 1922 Jour. Bact., **7**, 537.
Rahn, Otto. 1932 To appear in Jour. General Physiology.
Reichenbach, H. 1911 Zeitschrift für Hygiene, **69**, 171.
Wilson, G. S. 1922 Jour. Bact., **7**, 405.