A CHEMICAL EXPLANATION OF THE VARIABILITY OF THE GROWTH RATE

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The growth properties of a cell are essentially concentrated in the chromosomes. These are believed to consist of subunits, the genes, and each of these genes seems to be the carrier of a special property of the organism. Each gene must therefore be chemically different from all other genes of the same cell, because each brings about a different chemical (or physical) reaction. Few organisms are known which have duplicate or triplicate chromosome complexes. Multiplication of cells seems to be brought about primarily by the reactions of these genes which may be considered growth catalysts.

Division of a cell is preceded by a division of all chromosomes which means a division of all genes. This doubling of the genes is the final step of an elaborate synthetic process, but it is the only step which we can observe.

Since all genes in one cell are chemically different and since they do not appear to react with one another, it is probable that the doubling of each gene in a cell is independent of the doubling process of the other genes, and that we may consider the rate of doubling of one gene separately.

If we have a large number of uniform cells, containing the same number of molecules in the same arrangement, it may be that the same gene in all these cells will double at the same moment. With this assumption, all identical cells should multiply at the same moment. It seemed more probable to the author, however, that the doubling of a gene, being a chemical process, would follow chemical laws. Just as molecules in a solution do not all react at the same time, but follow a definite order, so the corresponding genes in a number of identical

cells may not double at the same moment, but follow a definite order. The simplest assumption, in analogy to the mass law, is that the rate of doubling is proportional to the number which have not yet doubled.

With this assumption, identical cells would not all double at the same time; some would divide faster than others, but this difference would be a matter of chance only, and would not be inheritable.

It is possible to compute the order in which cells would multiply if the latter assumption were true. Let us assume for the start a theoretical organism with only one gene. Let a be the number of identical cells, and let m be the fraction of genes doubling per unit time. The number of cells in this case is the same as the number of genes. We then find:

	Cells with gene unchanged	Cell with gene doubled
At the start	a	0
After 1 time unit	$a(1-m)$	$a \cdot m$
" 2 " "	$a(1-m)(1-m)$ $= a(1-m)^2$	$am + am(1-m)$ $= am(1+1-m)$
" 3 " "	$a(1-m)^3$	$am[1+(1-m)+(1-m)^2$
" t " "	$a(1-m)^t$	$am[1+(1-m)+(1-m)^2+$ $\ldots\ldots+(1-m)^{t-1}]$ $= a[1-(1-m)^t]$

This deduction implies something analogous to a monomolecular reaction because it assumes that m is independent of the ratio gene: food, or that the concentration of food within the cell is not changed by the doubling of the gene. For well nourished cells, this assumption may be correct. The growth rate of bacteria is known to be independent of the food concentration above a fairly low limit.

If *two* genes must double before cell division can occur (the two genes reacting independently of each other), they would offset each other's chances for cell division to a certain extent, since one gene might react very early in a certain cell and the other very late. This would be an extreme case; the probability of the event that both molecules have reacted can be computed. If the probability that the first gene has doubled in a given time is P_1 and the probability that the other has doubled, is P_2, then the probability that both genes in the

same cell have doubled is $P = P_1 \cdot P_2$. The probability that the first gene has doubled after the time t was found to be

$$P_1 = 1 - (1 - m)^t$$
$$= 1 - q^t$$

if we substitute the continuously reappearing term $1 - m = q$; q is the proportion of not-reacting genes per unit time.

The probability that the other gene (doubling at the rate of n genes per unit time) has reacted, is

$$P_2 = 1 - (1 - n)^t$$
$$= 1 - q'^t$$

The probability that both genes have reacted in the same cell is $P = P_1 \times P_2$. This formula can be simplified considerably by assuming that though these two genes multiply independently, they multiply at the same rate which means $m = n$, or $q = q'$. The probability that both genes have reacted in the same cell is then

$$P = (1 - q^t)^2$$

For any number of genes per cell, g, we would get the probability that all genes have reacted in the same cell, as

$$P = (1 - q^t)^g$$

This formula includes the assumption that all genes multiply at the same rate.

The computation of theoretical cases of this kind is easy. P, *i.e.* the probability that all genes are doubled, or that a cell can multiply, if plotted as a function of time, is a sigmoid curve. From this we can compute the probability that a cell will double at a certain time. This computation has been carried through for several different rates of doubling, and for different numbers of genes. In Tables I and II are given the percentages of cells which would multiply at successive time units; they represent frequencies or probabilities.

Fig. 1 (for $q = 0.5$) shows how this frequency is affected by the number of genes. If the first cases, with very few genes, are disregarded, the curves differ mainly in their relative position, in their distance from the zero point, *i.e.*, in the time elapsing before the first cell division occurs; this time increases with the number of genes. There is little

difference in the general shape of the curves. Fig. 2 shows the curve for eight genes placed over that for 128 genes, and the difference is very slight.

TABLE I

Percentage of Cells Doubling per Unit Time

	No. of genes per cell						
	1	2	5	10	50	100	500
When 90 per cent of all genes of each kind double per unit time ($q = 0.1$)							
During 1st time unit	90.00	81.00	59.05	34.87	0.52	0	0
" 2nd " "	9.00	7.01	36.05	55.56	59.98	36.60	0.64
" 3rd " "	0.90	1.79	4.40	8.57	34.62	52.78	59.96
" 4th " "	0.09	0.18	0.45	0.90	4.38	9.62	34.57
" 5th " "	0.01	0.02	0.05	0.09	0.45	0.90	4.33
When 80 per cent of all genes of each kind double per unit time ($q = 0.2$)							
During 1st time unit	80.00	64.00	32.77	10.74	0	0	0
" 2nd " "	16.00	28.16	48.77	55.75	13.00	1.69	0
" 3rd " "	3.20	6.24	14.52	25.79	53.93	43.11	1.80
" 4th " "	0.64	1.28	3.14	6.13	25.38	40.42	43.13
" 5th " "	0.13	0.28	0.64	1.27	6.12	11.66	40.42
" 6th " "	0.02	0.03	0.13	0.26	1.26	2.49	11.50
" 7th " "	0.01	0.01	0.02	0.05	0.25	0.50	2.52
When 70 per cent of all genes of each kind double per unit time ($q = 0.3$)							
During 1st time unit	70.00	49.00	16.81	2.82	0	0	0
" 2nd " "	21.00	33.81	45.60	36.12	0.90	0	0
" 3rd " "	6.30	11.87	24.79	37.12	24.51	6.48	0
" 4th " "	1.89	3.72	8.82	16.13	41.19	37.87	1.71
" 5th " "	0.56	1.12	2.77	5.41	21.94	34.05	27.91
" 6th " "	0.17	0.33	0.84	1.67	7.87	14.55	39.73
" 7th " "	0.05	0.11	0.26	0.51	2.50	4.87	20.20
" 8th " "	0.02	0.03	0.08	0.16	0.77	1.55	7.28
" 9th " "	0.01	0.01	0.02	0.04	0.22	0.43	2.15

The assumption that the doubling of genes obeys the mass law leads to the conclusion that uniform cells will not all multiply at the same moment, but show a definite variation of their growth rate. The range of this variation is practically unaffected by the number of genes. Relatively, the variability decreases with a larger number of

genes. From Table II it can be seen that in each case, about eight time units are required for the doubling of 98 per cent of all cells. But an organism with eight genes will start multiplying after the first time unit has elapsed, and the total time required for 98 per cent of all cells to multiply is nine time units, while an organism with 1000 genes will

TABLE II

Order of Growth
Computed for q = 0.5
Percentage of Cells Doubling per Unit Time

Time units	No. of genes per cell								
	1	2	4	8	16	32	64	128	1000
1	**50.00**	25.00	6.25	0.39	0	0	0	0	0
2	25.00	**31.25**	25.14	9.62	1.00	0.01	0	0	0
3	12.50	20.31	**26.98**	24.35	10.81	1.38	0.02	0	0
4	6.25	11.33	18.63	**25.31**	23.80	11.29	1.59	0.03	0
5	3.12	5.96	10.82	17.90	**24.56**	23.52	11.50	1.69	0
6	1.56	3.05	5.83	10.59	17.56	**24.21**	23.39	11.60	0
7	0.78	1.54	3.01	5.76	10.47	17.39	**24.03**	23.31	0.04
8	0.39	0.78	1.54	3.00	5.73	10.43	17.31	**23.96**	1.96
9	0.20	0.39	0.77	1.53	2.99	5.71	10.40	17.27	12.16
10	0.10	0.19	0.39	0.77	1.53	2.98	5.70	10.37	23.44
11	0.05	0.10	0.20	0.39	0.77	1.53	2.99	5.71	**23.80**
12	0.02	0.05	0.09	0.20	0.39	0.78	1.53	3.01	17.20
13	0.01	0.02	0.05	0.09	0.20	0.38	0.77	1.51	10.00
14	0.00	0.02	0.02	0.05	0.09	0.20	0.38	0.77	5.40
15*			0.02	0.02	0.05	0.09	0.20	0.38	2.98
16			0.01	0.02	0.02	0.05	0.09	0.20	1.50
17				0.01	0.02	0.02	0.05	0.10	0.77
18					0.01	0.02	0.02	0.05	0.38
19						0.01	0.02	0.02	0.20
20							0.01	0.02	0.10

* All data for more than fourteen time units have not really been computed, but are derived from the parallelism of the other curves.

show practically no multiplication during the first seven time units, and a total of fifteen time units is necessary for 98 per cent of all cells to multiply. With the first organism, variability spreads over $\frac{8}{9}$ = 89 per cent of the total time, with the second organism only over $\frac{8}{15}$ = 53 per cent.

Even with 1000 genes, the "variability" due to chemical laws is considerable, and should be quite conspicuous in all experiments on growth

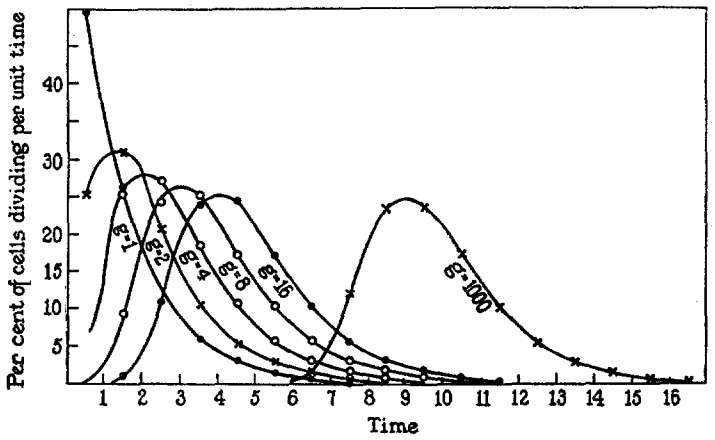

FIG. 1. Percentage of cells dividing in successive time units when the number of gene-type molecules per cell varies from 1 to 1000. (data computed for $q = 0.5$)

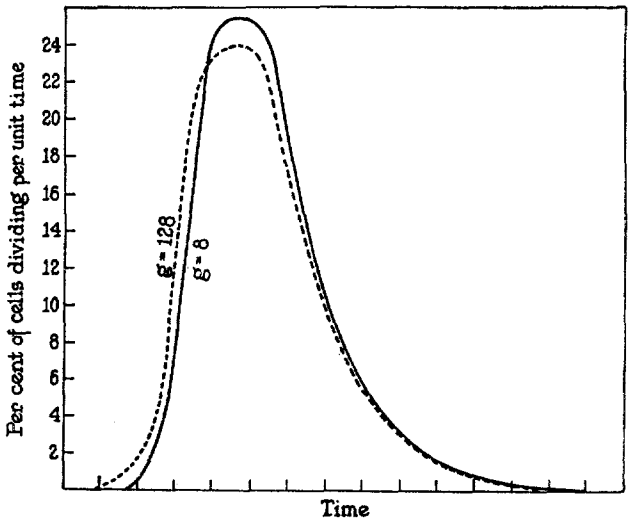

FIG. 2. Two curves for different numbers of genes from Fig. 1 drawn one over the other to show their similarity.

rates. With an organism possessing many genes, the growth rate should be less variable, relatively speaking, than with a simpler organ-

ism possessing only a few genes. This seems contrary to the author's "feeling." He would have reasoned that an organism possessing many genes, and therefore many properties, would show a wider range of variability of growth rate, being subject to more chances.

Another interesting fact is that the curve remains asymmetrical. Asymmetry is to be expected when the number of genes is very small, but the data in Tables I and II show a great constancy of the asymmetry even when the number of genes reaches 1000, and the same would be the case with a million genes.

EXPERIMENTAL EVIDENCE

In the introductory paragraphs, two possibilities for the doubling of genes in uniform cells were mentioned: either the same gene in all identical cells doubles at the same moment, or the doubling follows the mass law. The consequences of both assumptions have been discussed theoretically. It remains to compare both theories with the experimental evidence.

With bacteria, the customary method of measuring growth by plating is not applicable because we must obtain data on individual cells, while the plate count gives averages only. In a rapidly growing bacterial culture, we have all different stages of cell division simultaneously, and for the measurement of the order of growth, we must have all cells at the same starting point. Thus, only direct microscopic observation is likely to give us the desired data.

In one case only can the plating method be used, namely, in the germination of spores. We may consider all spores at the same stage of development. The order of spore germination has been measured by the plating method by Eijkman (1912–13), and he found the remarkable fact that the spores germinate in logarithmic order. There may be a very short period in which spores show no germination but as soon as they start, the rate of germination is proportional to the number of ungerminated spores. Fig. 3 shows the percentage of spores germinating per unit time.

The direct microscopic measurement of the generation times of a number of individuals is possible by using the agar hanging block (Orskov, 1922). K. A. Jensen (1928) gives a few data on the growth of *Bacterium coli*, but the number of individuals was too small and the

time intervals of 30 minutes too long to use these results for our purpose.

Mr. C. D. Kelly, together with the author (1931), has made a number of measurements of the growth rate of *Bacterium aerogenes* and *Bacillus cereus*. The data obtained with the latter organism cannot be applied here because spore-forming bacilli, at the period of fastest growth, sometimes do not form the cell walls separating the two new cells from one another, and no other indicator of the accomplished cell division was available. With *Bacterium aerogenes*, ten complete experiments were performed. These must be divided into four groups because the mode of the growth rate was not the same in all of them, and for our purpose, only such experiments can be summarized which

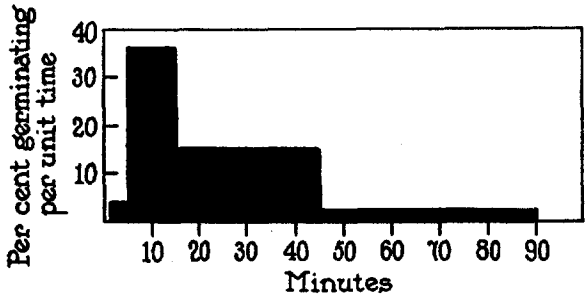

FIG. 3. Percentage of bacteria spores germinating in successive 10 minute periods.

show the same mode. Table III shows the three largest groups, with 733 fission times observed under the microscope. All three sets of data are skewed to the left.

These observations include usually four generations. If the variations observed in the growth rates are due to the chemical mass law, then they are a matter of chance, and the cells deriving from very slow and from very rapid fissions should have the same chances as all others. This is actually the case. No inherently slow or rapid growing strains were observed, and a rapid division was sometimes succeeded by a slow one, more commonly by an average one.

Table IV shows the fission times of the progeny of the 109 fastest growing individuals and of the 48 slowest growing individuals.

The same experiment was carried out with a yeast, *Saccharomyces*

ellipsoideus. The intervals were again 5 minutes. Except for the longer time required, the results are quite similar, as the two series in Table V show.

Here again, the frequency curve is distinctly skewed. In Series A, the six cells (2.7 per cent) dividing at the very rapid rate of 45 to 50

TABLE III

Frequency of Fissions of Bacterium aerogenes
(Number of Fissions Observed in Successive Time Intervals of 5 Minutes)

Time interval No...	2	3	4	5	6	7	8	9	10	11	12	13	14	Over 14	Total
Feb. 17.....					2	13	12	7	7	2	1				44
" 24.....			2	3	4	20	16	11	2	1		1			60
Mar. 3.....		1	3	12	20	38	19	18	8	4	1	1		1	126
" 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
Percentage...	—	0.3	3.4	7.8	13.0	30	20.7	13.9	6.2	2.5	0.6	0.6	—	1.5	
Mar. 2.....	1		3	19	22	21	10	5	2	1					84
" 6.....			4	12	30	19	15	1		2	1				84
Total.....	1	—	7	31	52	40	25	6	2	3	1	—	—	—	168
Percentage...	0.6	—	4.1	18.5	31.0	23.8	14.9	3.6	1.2	1.8	0.6	—	—	—	
Mar. 17.....		6	23	43	30	9		1							112
Nov. 12.....			13	60	24	3									100
" 14.....			5	13	4	5	2	1							30
Total.....	—	6	41	116	58	17	2	2	—	—	—	—	—	—	242
Percentage...	—	2.4	17.0	48.0	24.0	7.0	0.8	0.8	—	—	—	—	—	—	—

minutes (10th interval) are the progeny of one cell and the two cells (0.9 per cent) dividing between 60 and 65 minutes (13th interval) are sister cells to these. Here was a distinctly inherent faster growth rate noticeable which is not due to chemical variation; it is the only example observed in this investigation.

No data could be found which give the actual rate of multiplication of protozoa, though probably some such experiments obtained under uniform conditions are available somewhere. The same pertains to data on algae.

The experimental evidence with bacteria and yeasts is in accordance with the assumption that the doubling of genes follows a chemical law. The variability curves are skewed to the left, as this assumption demands. The variation is relatively smaller with yeast than with

TABLE IV

Frequency of Fissions of the Most Rapidly and the Most Slowly Reproducing Individuals of Bacterium aerogenes. (Number of Fissions in Successive 5-Minute Intervals)

Time interval No.	2	3	4	5	6	7	8	9	10	11	12	13	14
109 fastest cells	0	3	7	18	14	36	10	10	4	2	1	0	4
48 slowest "	1	2	9	11	2	4	5	2	9	3	0	0	1

TABLE V

Variability of Budding Times in Yeast. (Percentages of Budding Cells)

Time interval No.	10	11	12	13	14	15	16	17	18	19	20	21
Series A 224 data	2.7	—	—	0.9	—	2.7	6.3	19.6	21.9	13.0	9.4	8.5
" B 194 "	—	—	—	—	—	—	—	—	—	—	—	2.1

	22	23	24	25	26	27	28	29	30	31	32	33
Series A	1.8	0.9	0.9	2.2	4.0	2.7	2.7	—	—	—	—	—
" B	11.4	8.2	16.5	19.6	10.8	8.7	1.1	3.1	—	1.1	1.1	1.1

bacteria which corresponds to the requirement that the spread of variation is relatively smaller as the number of genes increases. There is no proof that a yeast has more genes than *Bacterium aerogenes*, but it appears probable. The variation between fast and slow growing cells is not inherent, but merely a matter of chance, as the theory demands.

It might be claimed, on the other hand, that the experiments do not contradict necessarily the other assumption that uniform cells multiply at the same moment, and that any differences in growth rate are caused

by differences in the cells or in environment. However, it seems that the great differences found in the growth rates of sister cells lying side by side in the same medium can hardly be accounted for in this way.

Chemical Interpretation of the Variability of the Growth Rate

The above discussion intends to show that what we ordinarily call variability of growth rate is not variability in the common sense of the word, but the result of the working of chemical laws. For the sake of simplicity, the genes have been supposed to be the simplest units of the chromosomes. This assumption is not necessary. The calculation is just as correct if we assume each gene to consist of a number of molecules, which, owing to their peculiar properties, shall be called gene-type molecules. As soon as we designate the letter g to mean the number of gene-type molecules per cell, and not the number of genes, the formula for the variability includes no assumption about the size of genes and the number of molecules in one gene.

Several assumptions have been made in this discussion which might be doubted. The entire deduction is based upon the assumption that in uniform cells, under uniform conditions, the molecules in the various cells react as if all cell contents formed a continuous medium. This assumption cannot be proved. It can only be stated that the order of death of bacteria is in agreement with this assumption. If this can be shown to be definitely wrong, then the entire deduction is wrong.

It has been further assumed that the reaction leading to the doubling of the gene-type molecules is monomolecular. This makes the calculations of the formulas easy. But if the reactions were of a higher order, the general principle of the "order of growth" would not be altered; only the formula and the curves would become much more complicated. There would be still a great difference in time between the first and the last completion of the doubling of all genes.

It has further been assumed that a cell divides as soon as all genes are doubled. Originally, the term "cell division" was just used as a simpler, shorter term for the doubling of all genes in one cell, but when we come to experimental proofs, it assumes a more definite meaning. If it takes 2 hours for all genes in one cell to double, and then 20 minutes longer before cell division can be actually observed, the frequency for the experimental order of growth lags 20 minutes behind the calculated curve. This inaccuracy does not change the type of curve, nor the principle of the "order of growth." But it prevents drawing far-reaching conclusions.

It has also been assumed that all gene-type molecules divide at the same rate. There is no evidence to prove or disprove this. If the rates of division were very different, this would make the problem much more difficult mathematically but

would not affect the principle under discussion, and would still give a large and experimentally measurable range of variability.

One further assumption is implied, namely, that a gene-type molecule, after having doubled, "waits" for all others to double, and does not start to double again until the chromosomes have doubled. Otherwise one gene might double a second time before another has doubled for the first time, and the balance of chromosomes would be upset. There must be some regulatory mechanism in the cell to prevent this. The nature of this mechanism is absolutely unknown. This regulation must exist in the cell even if the theory here presented should prove to be wrong.

The mathematical calculation had to be over-simplified to bring out the principle that variability of the growth rate is a chemical necessity. Of the many assumptions made for mathematical treatment, only the application of the mass law to molecules in a number of uniform cells is essential for the theory. This is the same assumption which the author has used to account for the differences in the order of death between bacteria and larger organisms (Rahn, 1929, 1930, 1931). All other assumptions do not affect the principle of the theory, but only the ease and accuracy of its mathematical formulation.

It has already been pointed out above that with unicellular organisms, the relative spread of variation (relative to the average time required) is largest with the simplest organisms. This might be used as a means to compute the number of gene-type molecules in unicellular organisms if we had very accurate data on the variability of the growth rate, and if cell division would take place at the same moment when the last gene has doubled.

We have to take the ratio of the times required to reach two definite points of the variability curve. We shall choose the moment when 10 per cent of the cells have doubled, and the moment when 90 per cent of the cells have doubled. The general equation was

$$P = (1 - q^t)^g$$

In the first case, we have $P = 0.1$; in the second case, $P = 0.9$

The above formula gives $P^{\frac{1}{g}} = 1 - q^t$

$$q^t = 1 - P^{\frac{1}{g}}$$

$$t = \frac{\log \left(1 - P^{\frac{1}{g}}\right)}{\log q}$$

$$t_{10} = \frac{\log\left(1 - 0.1^{\frac{1}{g}}\right)}{\log q}$$

$$t_{90} = \frac{\log\left(1 - 0.9^{\frac{1}{g}}\right)}{\log q}$$

The ratio between these two times is independent of the growth rate, q:

$$\frac{t_{90}}{t_{10}} = \frac{\log\left(1 - 0.9^{\frac{1}{g}}\right)}{\log\left(1 - 0.1^{\frac{1}{g}}\right)}$$

It is difficult to determine g from this equation, but we can easily compute the ratio for any definite g. We find:

$$\text{for } g = 10,\ r = \frac{t_{90}}{t_{10}} = 2.87$$

$$\text{for } g = 100,\quad r = 1.83$$

$$\text{for } g = 1000,\quad r = 1.58$$

The difference between the last two ratios is so small that it seems rather hopeless to determine the number of gene-types by this method if the number is high, considering also the inaccuracy in determining experimentally the order of growth. For organisms with less than 100 genes—if such organisms should exist—this method might suffice for an approximation provided that completely homogeneous material can be obtained for the purpose. Any variability in the organisms or in the environment will tend to increase t_{90} and to decrease t_{10}, *i.e.*, it will increase the ratio $\frac{t_{90}}{t_{10}}$ and make the number of genes appear smaller than it really is.

The three sets of data on *Bacterium aerogenes* in Table III give the following values for $\frac{t_{90}}{t_{10}}$: 1.95; 1.85; 1.65.

From the two yeast experiments, this quotient assumes the values, 1.46 and 1.28. While we could draw no conclusions regarding the number of gene-type molecules per cell, we might conclude that yeast has a larger number of genes than *Bacterium aerogenes*.

There is one more agreement between theoretical calculation and experiment. Following a suggestion by Crozier (1931, footnote p. 20), it seemed probable that the skew of the theoretical frequency curves might be caused by the orderly gradual change of the velocity of

growth, or of the velocity of the reaction of the gene-type molecules. In this case, a normal, *i.e.*, symmetrical frequency curve should be expected if the number of organisms multiplying per time unit is divided by the time unit before being plotted against time. In the theoretical cases as well as in the experiments with bacteria and yeast, the frequencies thus plotted proved to be quite symmetrical.

Growth Rates of Multicellular Organisms

With multicellular organisms, only the time required for several consecutive cell divisions can be measured, and it is not certain that this number is the same in each individual. For example, it seems doubtful to the author that all insect larvae of one species have the same number of cells. Besides, there is, with most higher organisms, a considerable amount of specialization of the cells, and different types of cells will probably multiply at a different rate. The simplest case where all cells are at least functionally alike is that of bacteria colonies. This is the only case that can be treated mathematically. Even there, we have to make the assumption that the average growth rate remains constant which will not be correct for the later stages of development.

A mathematical treatment may be possible, but the author did not succeed. However, a purely empirical calculation of the variation of the time required to complete three or ten cell divisions is simple. Table I has shown that for $q = 0.1$ and $g = 100$, the frequencies of cell division for the first five time units are: 0; 36.6; 52.78; 9.62; 0.90. Each of the cells born at these times has the same chances again during the second cell division, and it can be easily computed how the fission times of the offspring of the first 36.6 per cent will vary. The 52.78 per cent of the third time interval again multiply according to the same probability curve, and so do the 9.62 per cent and the 0.9 per cent of the other two time units. In this tedious, but simple way, the frequency distribution for any number of generations can be computed, as Table VI shows, and Table VII gives the result in per cents for ten generations of the above example. By these ten divisions, the cells have multiplied 1024-fold.

If an organism existed which consisted of ten generations = 1024 cells of uniform composition, the last row of Table VII would show the expected variation of time required for the development of this organ-

TABLE VI

Calculated Number of Cells Completing the 1st, 2nd, 3rd, 4th, and 5th Generation during Successive Time Units ($g = 100$, $q = 0.1$)

Time unit	No. of cells dividing per time unit																			Total cells
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	
Generation I	0	366	528	96	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1,000
" II	0	0	0	268	776	698	216	38	4	0	0	0	0	0	0	0	0	0	0	2,000
" III	0	0	0	0	0	196	852	1,384	1,050	404	98	16	0	0	0	0	0	0	0	4,000
" IV	0	0	0	0	0	0	0	142	834	1,954	2,400	1,688	732	208	42	0	0	0	0	8,000
" V	0	0	0	0	0	0	0	0	0	104	760	2,342	3,988	4,150	2,830	1,292	422	100	12	16,000

TABLE VII

Calculated Frequency (in Percentages) of the Times Required by Uniform Cells to Complete from 1 to 10 Generations ($g = 100$, $q = 0.1$)

Time units		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
After:	Total No.																	
1st generation	2 a	0	36.6	52.8	9.6	0.9												
2nd "	4 a				13.4	38.8	34.9	10.8	1.9	0.2								
3rd "	8 a						4.9	21.3	34.6	26.2	10.1	2.5	0.4					
4th "	16 a								1.9	10.4	24.4	30.0	21.1	9.1	2.6	0.5		
5th "	32 a										0.7	4.8	14.6	24.9	25.9	17.7	8.1	2.5
6th "	64 a												0.2	2.1	7.9	17.3	24.1	22.8
7th "	128 a														0.1	1.0	4.0	10.7
8th "	256 a																	0.5

Time units		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
5th generation	32 a	0.7	0.1															
6th "	64 a	15.0	7.2	2.6	0.7	0.1												
7th "	128 a	18.7	23.0	20.0	12.9	6.4	2.5	0.6	0.1									
8th "	256 a	2.0	6.1	12.9	19.3	21.4	17.8	11.2	5.7	2.2	0.7	0.2						
9th "	512 a		0.2	1.0	3.3	8.1	14.5	19.4	19.7	15.8	9.9	5.1	2.1	0.7	0.2			
10th "	1024 a				0.1	0.5	1.7	4.9	9.8	15.6	19.0	18.1	14.1	8.8	4.5	1.9	0.8	0.3

ism from a single cell. Table VII and Fig. 4 indicate that the frequency curve becomes flatter as the number of generations increases, but it also shows that the relative spread of variation decreases.

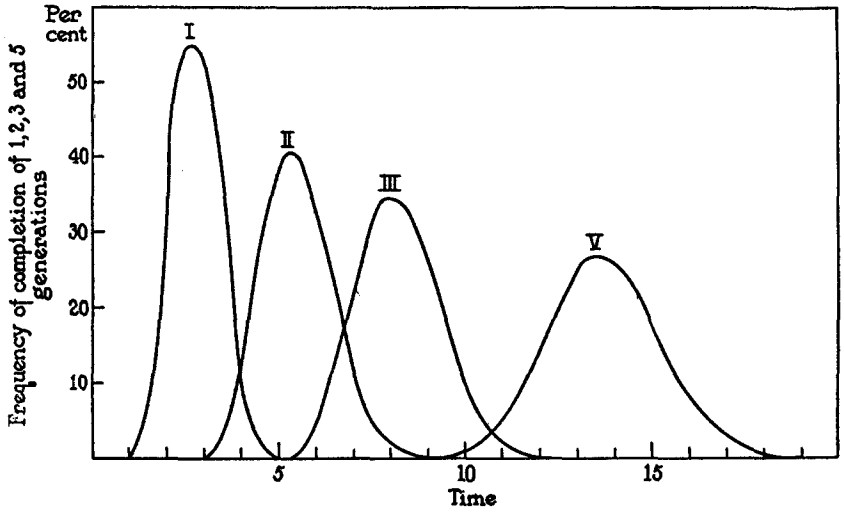

FIG. 4. Percentage of cells completing their 1st, 2nd, 3rd, and 5th division in successive time units.

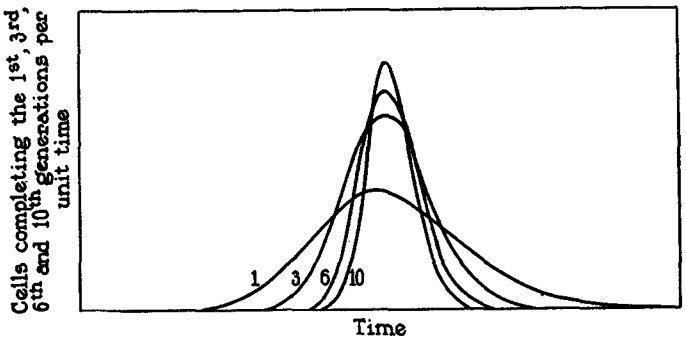

FIG. 5. Percentage of cells per unit time completing 1, 3, 6, and 10 generations, drawn to equal modal distances.

This is demonstrated graphically in Fig. 5 which presents four of the series from Table VII in such a way that the modes of all of them coincide. This required a different scale on the abscissa for each curve;

to keep the area of the curves uniform, the ordinates were enlarged in the same proportion as the abscissae were shortened. The figure shows very plainly how the relative variation decreases as the size of the "organism" increases from 1 to 1024 cells. It also shows that with an organism of this size, the variability of the growth rate should still be experimentally measurable.

An experiment with bacteria colonies verified this expectation. A number of nutrient agar plates were flooded with a very young culture of *Bacterium aerogenes* which had been transferred repeatedly at 3 hour intervals. The cells remaining on the agar surface after pouring off the excess liquid developed at 30° into colonies. At four different intervals, some of the plates were treated with formaldehyde to prevent further growth. The diameter of 100 colonies was measured for each

TABLE VIII

Variation in Diameter of Bacteria Colonies of Four Different Ages

Age of colonies	One division of the scale equals	Percentage of colonies having the following diameter of the scale														
		30		40		50		60		70		80	90	100	110	120
6½ hrs. old.....	0.8 μ	—		1		5		6		24		31	19	8	3	3
9¾ hrs. old.....	8.0 μ	1	1	9	20	32	21	11	3	1	1	—	—	—	—	—
13½ hrs. old.....	23.3 μ	—	—	—	4	8	46	23	16	2	—	—	—	—	—	—
16½ hrs. old.....	46.6 μ	2	—	11	53	22	9	3	—	—	—	—	—	—	—	—

of the four stages of development, and the results arranged by frequencies, are given in Table VIII.

The scales have been chosen so that the entire range of variability can be compared in one table, and it is evident that with increasing age, the relative range decreases. That is all that could be proved by this experiment, and it agrees with the theoretical deduction of the preceding pages.

The general principle that increase of the number of generations, *i.e.*, increase of the number of cells of an organism, decreases the relative range of variability is quite evident from a number of data on multicellular organisms. Fig. 6 shows a few such data of different organisms drawn in the same manner as Fig. 5, namely so that all modes coincide. The data used are the gestation period of rabbits,

from 200 data kindly provided by Mr. R. B. Hinman of the Animal Husbandry Department of Cornell University, and the gestation period of cows, after data from Wing (1899). For comparison, one yeast experiment from Table V, and the last group from Table III of *Bacterium aerogenes* are included.

Data on the hatching times of insect eggs, also on other stages of insect development, have been furnished by Sanderson and Pears (1913), Parker (1930), and many others, but the eggs do not represent single

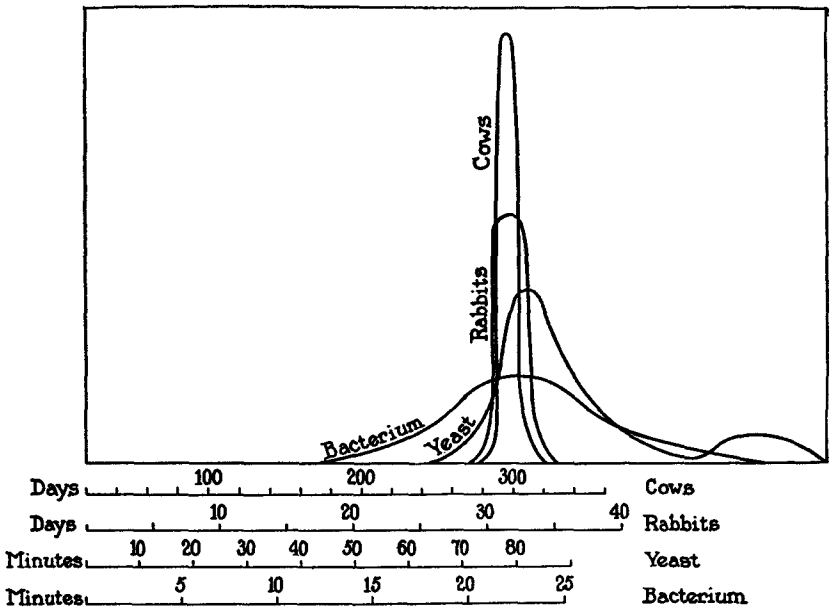

FIG. 6. Variability of the growth rate of a bacterium and a yeast, and of the gestation period of rabbits and cows, all drawn to equal modal distances.

cells, and therefore do not fit into Fig. 6. Most of the frequency curves plotted from these data are skewed to the left, but there are a number of exceptions. The curves on the gestation period of cows and rabbits, are practically symmetrical. Data on the "gestation period" of insects could not be found.

The question arises whether this difference in the growth rate of identical cells, if it is due to chemical laws, should be called "variability." It seems to be the practice of limiting this term in technical

language to differences in the composition of the organism which are too slight to be detected by other means. When the cause of different behavior of similar organisms is known, it is not usually termed "variability."

In this case, the cause of "variability" seems to be the mass law. The cause is known, but it cannot be eliminated experimentally. We cannot make 100 uniform bacteria cells divide all at the same time any more than we can make all sucrose molecules of a sugar solution invert at the same moment. In this respect, the cause of variation of the growth rate is different from the causes of all other variations.

SUMMARY

The general belief that uniform cells under uniform conditions will all multiply at the same moment implies that the smallest units of the chromosomes, *i.e.*, either the genes or the molecules of which the genes are composed, all double at exactly the same moment in all cells.

Since the doubling of chromosomes is a synthetic chemical process, it seems more probable that it would follow chemical laws. With the assumption that the corresponding molecules in a number of uniform cells obey the mass law in their process of doubling, a definite order in the multiplication of identical cells is established which can be formulated mathematically for the simplest case. This is the same assumption which the author has used to account for the differences in the order of death between bacteria and higher organisms.

This theory demands a great variability of the growth rate of uniform cells, so great that it must be experimentally measurable even for cells with a million molecules to the chromosome.

The theory demands further that the frequency curve of cell divisions plotted for successive time intervals, be skewed to the left, and that the relative range of variation become smaller as the number of genes or gene-type molecules increases.

Experiments on the growth rate of *Bacterium aerogenes* and *Saccharomyces ellipsoideus* showed regularly a frequency curve skewed to the left. The yeast had a relatively narrower range of variability than the bacterium.

Even with multicellular organisms, theoretical calculations show a range of variation of the growth rate from the egg cell which should still be measurable though it decreases relatively with the number of cells produced. An experiment on the size of bacteria colonies at different ages of development agreed with the theory.

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