Individuality of Bacteria

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WHEN WE READ about a cure made with only one patient, or about feeding experiments with only two cows, we may hesitate to accept such results as representative. The individuality of the very few organisms used may differ from the normal average. With the customary bacteriological technique, the study of a single cell is an exception. Practically all experiments are started with thousands or millions of bacteria. Considering the very small size of bacteria, one may wonder whether they have any individuality.

For a discussion of this type, it is necessary to define individuality. It may be described as the difference (of action, behavior, or morphology) between single individuals of the same species.

Molecules have no individuality. Molecules of a pure compound are absolutely alike, and react identically. Differences in the *time* of reaction are due to their distribution in space, not to differences between the molecules themselves. A crystallized virus or a bacteriophage consists of only one molecule (Jordan, 1938), therefore all crystallized viruses are exactly alike, and can have no individuality. Individuality exists only with organisms consisting of many molecules. Bacteria consist of many molecules. Hand (1933) has shown that a yeast cell contains about 20,000 molecules of catalase. A yeast cell contains also several other enzymes, and other cell contents, and a cell wall. The number of molecules in the smaller bacteria is still large, and the number of molecules in a single cell cannot possibly be less than of the order 10^5 which does not include the inorganic matter.

If we consider the individuals of one certain species, they contain the same types of molecules; they all have the same kind of cell wall, the same protoplasm, the same enzymes, the same minerals, the same kind of water molecules. But each individual may have *different numbers* of these molecules, and that accounts for their individuality. Leonardo da Vinci wondered why no two leaves of the same mulberry tree were exactly alike; he could always find differences. If life is purely mechanical, if it is nothing but a sequence of chemical and physical reactions, then cell division and growth under carefully standardized conditions should be uniform. Standardization of organisms will be easiest when only a small volume is concerned. This reasoning speaks against any individuality of bacteria, as the smallest organism.

However, bacteria display their individuality whenever we take pains to observe individuals.[1] As early as 1921, Henrici began a series of publications showing that the single cells of a pure culture are not at all alike in their morphology. His studies were compiled in a monograph in 1926. A great physiological difference was also observed by Kelly and Rahn (1931) and Rahn (1932) with the growth rate of individual bacterial cells. This was done by spreading a diluted suspension of young bacteria on agar surface, cutting small blocks out of this agar, and attaching these little blocks onto sterile coverglasses so that the bacteria touched the coverglass. The coverglass was then sealed to a hollow-ground slide, and with this agar hanging block, the multiplication of each cell could be followed easily with the oil immersion lens. The bacteria were drawn every five minutes with a *camera lucida,* special attention being given to the cross membranes indicating cell division. It was possible to measure the tempo of four to six cell divisions, and since one field of the microscope at the start usually contained several cells, one experiment yielded the measurement of a good many cell divisions.

The entire procedure was done in an incubator room at 30° C. Most of the work was done with *Aerobacter aerogenes,* some with *Bacillus mycoides,* and some with a yeast. The cells were so young that the lag phase was entirely avoided. Figure 1 shows the record of one hanging block, and Table 1 gives the accumulated records of all experiments with *Aerobacter.* It will be seen that not all cells multiply at the same rate, some dividing between 10 and 15 minutes while the slowest require more than 70 minutes, the most frequent generation time being 25 to 35 minutes; 48 per cent of all ob-

[1] It has already been mentioned that viruses have no individuality because they consist of only one molecule. The writer does not consider viruses as living organisms because they cannot reproduce themselves. They are manufactured by the host cell, like enzymes or other cell proteins; they merely serve as the pattern after which the host cell, in its effort to duplicate the native proteins of the cell, mistakenly synthesizes new virus molecules.

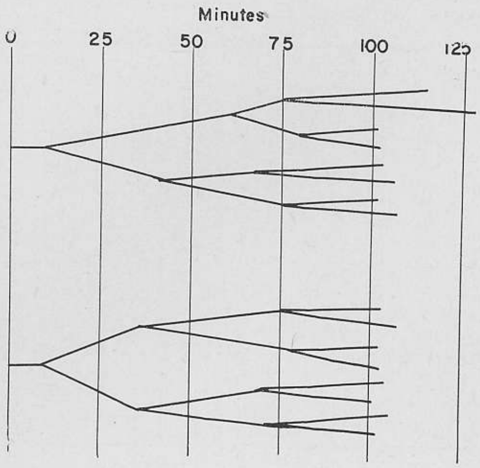

Fig. 1.—Single record of Aerobacter aerogenes

served cells divided at this time. The fluctuations with the other microorganisms were similar.

The variation in the rate of cell division is not a result of inheritance. The most rapidly dividing cells do not produce a rapidly dividing offspring. The division rate of their offspring is average. The same is true with the offspring of the slowest cells.

A cell cannot divide until each chromosome has divided, and this again depends upon the duplication of all genes in the cell. Duplication is a chemical reaction and is ruled by chemical laws. Each type of gene is different from all others, and therefore may have a different division time. (A gene consists of only one molecule (Jordan, 1938.) As we are dealing with different types of molecules, we cannot apply the law of mass action. There is no mass. The mass law is the law of chance applied to very large numbers of molecules. Chemical reaction is governed by the chance of collisions. If the chance for gene Number 1 is that 50 per cent of them double in the first 5 minutes,[2] and the chance for gene Number 2 is the same, then the chance that both genes in the same cell are doubled in 5 minutes is $0.5 \times 0.5 = 0.25 = 25$ per cent. The chance that 3 genes of the same cell double in 5 minutes is $0.5^3 = 0.125$, and so forth. It is possible, by trial and error, to approximate the frequencies of Table 1, and thereby obtain a conception of the number of genes per bacterial cell. Such a method can yield only a rough estimate since other unknown factors may enter. The

[2] Finney and Martin have calculated from the data of Table 1 that 48 per cent of all genes of *Aerobacter* double in each 5-minute period.

Table 1.—Aerobacter aerogenes

	Minutes													
	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75
Frequency of fission times of 634 cells	1	7	59	72	152	154	92	53	22	11	4	2	0	5
Progeny of the 109 most rapid cells	0	3	7	18	14	36	10	10	4	2	1	0	4	----
Progeny of the 49 slowest cells	1	2	9	11	2	4	5	2	9	3	0	0	1	----

estimate by this method suggested about one hundred genes for *Aerobacter,* or a smaller number. This result seems low since Demerac (1935) mentioned that for the chlorophyl mechanism alone of the corn plant, 71 genes had been found necessary. The requirement of genes for the entire plant must be very much larger.

However, this estimate for *Aerobacter* is probably still too high, for Finney and Martin (1950), who applied specialized mathematics to the data shown in Table 1, came to the conclusion that *Aerobacter* contains only 29$\pm$ 3 genes per cell. This seems a very small amount indeed since the genes are believed to be responsible for the inheritance of so many properties: cell form, flagellation, cell wall, protoplasm, and all the enzymes which *Aerobacter,* as faculative anaerobe, must have, since it is capable of producing several different acids, alcohols, and hydrogen as well as carbon dioxide from glucose. The alternative is plasma inheritance which seems to be well supported by experiments with protozoa.

If this explanation by the law of chance is correct, we should expect a relatively large spread of the generation time when few genes are concerned. A graphic comparison was made by using the average multiplication rate as the basis of 100, and calculating the frequency of deviation of the various species in percentages of their basis of normality. According to theory, the range should decrease when the number of genes per organism increases. Yeasts should have a relatively narrower range than bacteria, and Figure 2 shows this to be true. Vertebrates should have a relatively narrower range

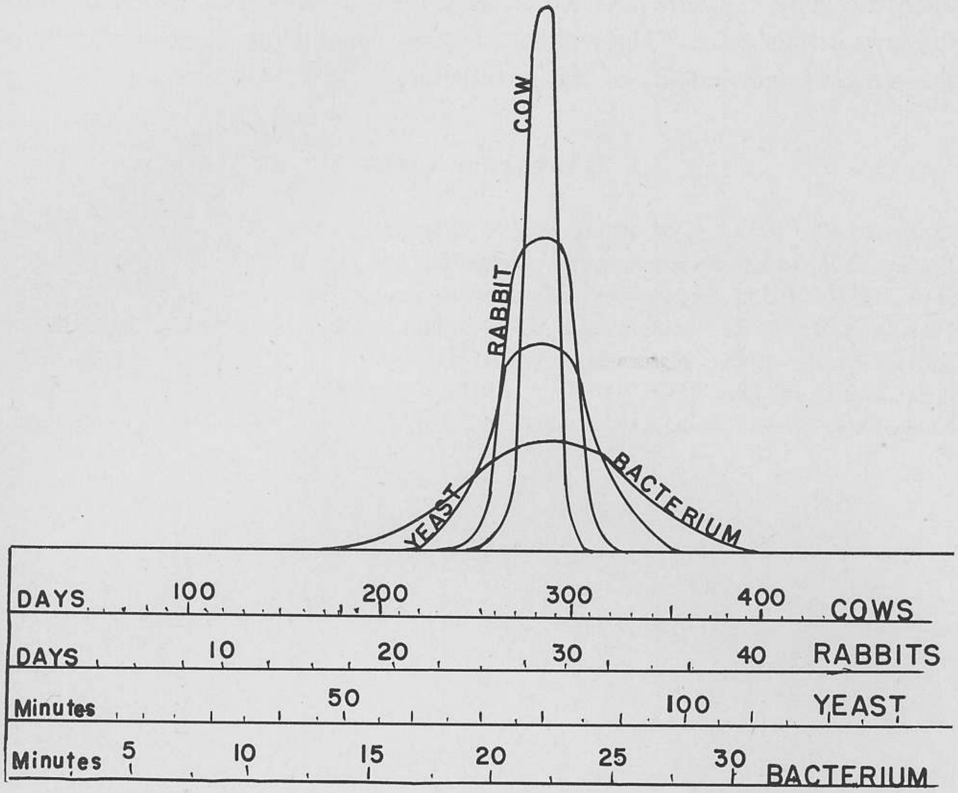

Fig. 2.—Range of individuality; frequency dis tribution of the rate of multiplication.

than unicellular organisms, and the range of the gestation period of cows should be narrower than that for rabbits. This is also shown to be the case in Figure 2. The width of the range is a measure of the range of individuality, as this word is defined by the above experiments.

Summary

The individual cells in a pure culture of bacteria or yeasts are not exactly alike. Their morphology may differ widely as Henrici has shown; their generation time also shows great differences under identical conditions. The differences in generation time are not inheritable. They are probably caused by the chance of duplication of each gene, and this chance can be approximated mathematically. Individuality is different from genetics. It is superimposed

upon the genetic factors, and has a mathematical foundation different from the laws of inheritance. The number of genes per cell can be estimated from the range of individuality of the growth rate.

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