THE BURST SIZE DISTRIBUTION IN THE GROWTH OF BACTERIAL VIRUSES (BACTERIOPHAGES)[1]

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Burnet (1929) invented a method for measuring the yield of virus from individual bacteria (burst size). It consists in diluting a suspension of infected[2] bacteria and incubating a large number of samples from the diluted suspension. Each sample should contain on the average less than one infected bacterium. Only a small fraction of the samples will then contain more than one infected bacterium. The samples are incubated until all bacteria are lysed, and then plated. Burnet did not apply this technique on a scale sufficiently large to indicate the distribution of individual burst sizes. Ellis and Delbrück (1939) and Delbrück (1942) obtained results which indicated an exceedingly wide variation of the burst sizes. However, these series of experiments were neither sufficiently large nor sufficiently accurate to give a clear idea of the burst size distribution. With some minor improvements in technique we have now succeeded in obtaining a fair approximation of the distribution.

MATERIALS

In this and the two following papers *Escherichia coli*, strain "B," and three different viruses were used. The bacterial strain and two of the viruses, alpha and gamma, have been used in previous work (Delbrück and Luria, 1942). The characteristics of the three viruses are summarized in table 1.

PROCEDURE

Virus is added to a growing culture of bacteria. The number of virus particles chosen is either somewhat below the number of bacteria, if single infection is desired, or in excess of the number of bacteria, if multiple infection is desired. A few minutes are allowed for adsorption. The mixture is then diluted with broth until it contains about 5 infected bacteria per ml. Forty to sixty samples of 0.05 ml each (containing on the average 0.25 infected bacteria) are then placed in small tubes and incubated. The distribution of the samples must be com-

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[2] We use the word "infection" of a bacterium by a virus to designate the fact that a bacterium has adsorbed a virus particle. We do not imply that the infecting particle necessarily grows. Thus, we will speak of "multiple infection" when several virus particles of the same strain become adsorbed on one bacterium, and of "mixed infection" when virus particles of different strains become adsorbed on the same bacterium. In all these cases very probably only one of the infecting particles grows. This use of the word "infection," though differing from medical usage, is etymologically correct and has been used previously in bacterial virus work.

pleted before the first burst has occurred. In the case of virus alpha, for instance, it must be completed within 13 minutes after the mixing of virus and bacteria. Incubation is continued until all bursts have occurred. In the case of virus alpha practically all bacteria are lysed 17 minutes after infection. At any time after the 17-minute period, therefore, the samples can be plated for plaque count. However, plating does not need to be done very soon after the burst since in this arrangement there is no danger of reinfection of other bacteria by the liberated virus.

For the purpose of plating we have adopted the agar layer technique, invented by Gratia (1936) and by Hershey *et al.* (1943). In this technique a few milliliters of melted agar of low concentration, containing the bacteria, are mixed with the sample to be plated. This mixture is poured on the surface of an ordinary

TABLE 1

Characteristics of the viruses

VIRUS	SIZE*	STRUCTURE*	PLAQUE SIZE	CONSTANT PERIOD	AVERAGE BURST SIZE
				min	
alpha.....	50 mμ	round head, slender tail 150 mμ long	medium	13	180†
gamma.....	65 × 80 mμ	oval head with internal structure, straight tail 120 mμ long	small	21	135
delta.....			large	13	300

* From electron microscope pictures (Luria, Delbrück, and Anderson, 1943).

† The burst size for alpha given here is higher than the value given previously (Delbrück and Luria, 1942). The new value is based on an extensive series of growth experiments run in parallel with the single burst experiments.

nutrient agar plate. The mixture distributes itself uniformly over the plate in a very thin layer and solidifies immediately. We used about 20 ml of 1.3 per cent agar for the lower layer and 1.5 ml of 0.7 per cent agar for the superimposed layer on petri plates of 7 cm diameter. With this technique 60 samples can be plated in about 20 minutes. The plaques are well developed and can be counted after 4 hours' incubation.

For our present purpose the chief advantage of the agar layer technique is that the entire volume of each sample can be plated with ease. In the usual technique, in which the sample is spread over the surface of the plate with a bent glass rod, not more than about 0.1 ml can be plated. It is difficult to transfer a sample of this size from a tube to a plate without losing part of it in the tube or in the transferring pipette. With the new technique the samples are also small but they are plated by adding several milliliters of liquid agar and pouring the resulting mixture. Thus only a very small portion of the sample remains in the tube.

RESULTS

Figure 1, lower half, shows the distribution of burst sizes for single infection by virus alpha. It summarizes the results of eleven similar experiments. In all, 620 samples were plated in these experiments, 150 of which showed bursts. On statistical grounds about 10 per cent of these must be ascribed to tubes in which two or more bacteria were lysed. The plaque counts from these accidental multiples will lie preferentially toward the high end of the distribution curve. The high end, therefore, may be slightly distorted.

Figure 1 shows that the burst sizes range from below 50 to over 1,000, with a

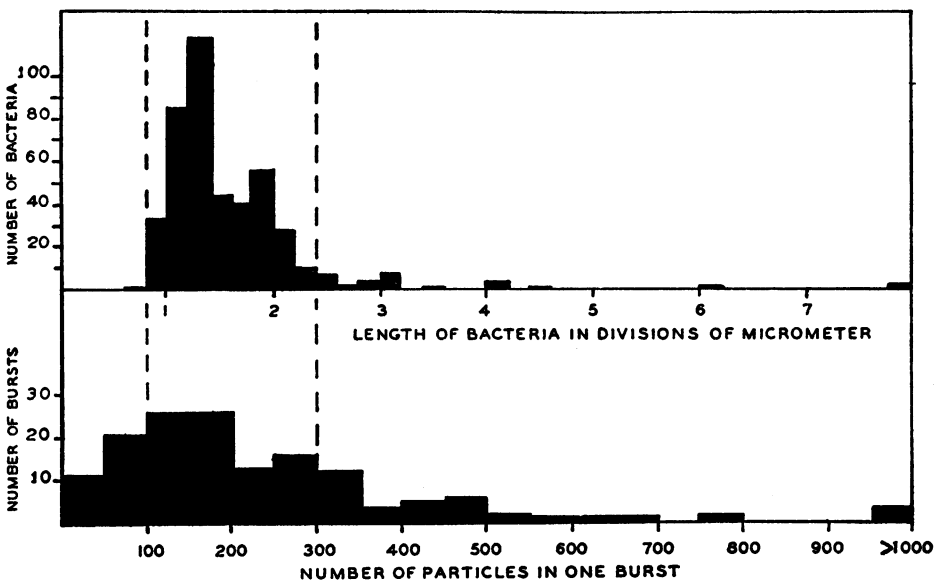

FIG. 1. DISTRIBUTION OF BURST SIZES (LOWER HALF) AND OF BACTERIAL SIZES (UPPER HALF)

The dotted lines are the limiting abscissae used in table 2. Single infection with virus alpha.

very broad maximum of the distribution around 180. Four bursts were smaller than 25. A part of this variation in burst size may be due to variations in the size of the bacteria. It is clear that in a growing culture the size of individual bacteria must range at least over a factor 2. A closer approximation may be obtained by micrometric measurements of the culture used for the burst size measurements.[3] Microscopic examination revealed that the bacteria were of uniform thickness and had a length distribution as represented in figure 1, upper half. The scales of the two distributions are chosen so that the maxima occur at the same abscissa. It will be seen at a glance that the burst size distribution is much wider than the distribution of bacterial sizes. A quantitative expression

[3] We are indebted to Miss Ann Martin for assistance in these measurements.

of this difference may be obtained by choosing two limiting abscissae and determining the percentage of individuals which fall within and outside these limits. Table 2 gives such a computation.

Ninety-four per cent of the bacteria fall within a range of size of a factor 3, against 54 per cent of the burst sizes. It is clear that the distribution of the burst sizes cannot be accounted for by the variation in bacterial size alone.

TABLE 2

Percentage of bacterial lengths and of burst sizes (virus alpha) which lie within corresponding limiting values

LENGTH OF BACTERIA	PERCENTAGE OF BACTERIA*	BURST SIZES	PERCENTAGE OF BURSTS†
(in divisions of the eye-piece micrometer)‡			
below 0.8	0.1	below 100	21
0.8–2.4	93.8	100–300	54
above 2.4	6.1	above 300	25

* 441 bacteria were measured.

† About 10% of these are accidental doubles.

‡ 1 division = 1.6μ.

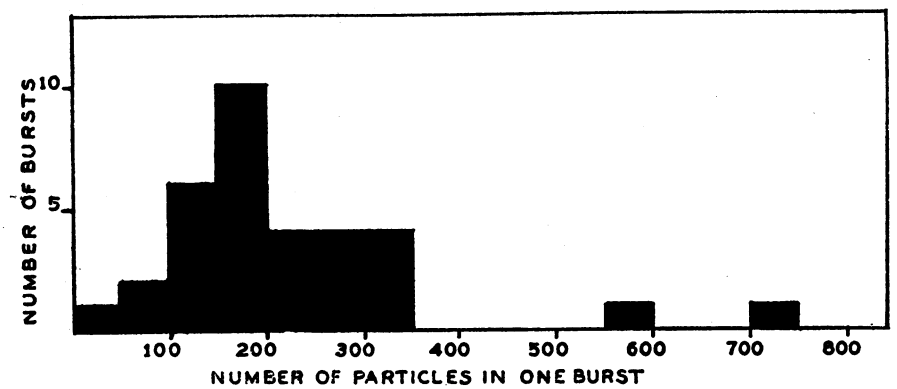

FIG. 2. BURST SIZE DISTRIBUTION FROM TWO EXPERIMENTS WITH FOURFOLD INFECTION BY VIRUS ALPHA

The large variation of burst sizes should be contrasted with the very small variation of the latent periods of individual bacteria. As mentioned above, bacteria infected with virus alpha are *all* lysed between 13 and 17 minutes after mixing. The processes leading from infection to lysis are therefore not subject to large fluctuations. One may infer that lysis is not determined by the attainment of a threshold number of completed virus particles. Rather the reverse may be true. Infection initiates a series of events which after a very definite time interval leads to lysis. This time interval is different for different strains of virus growing on the same host. The number of virus particles liberated upon lysis may then depend on the number which happened to have grown up

to that time. The large fluctuations in this number must be due to some feature in the mechanism of intracellular virus growth. In a general way large fluctuations in processes of this type ("autocatalytic") are easily accounted for (Delbrück, 1940).

Figure 2 shows the distribution of burst sizes from two multiple infection experiments with virus alpha. On the average each bacterium was here infected with four particles of virus alpha. In all, 120 samples were plated, showing 33 bursts. There is no significant difference between this distribution and that found for single infection by virus alpha.

Similar distributions, though with a higher average burst size, were obtained in experiments with virus delta, both in single infection and multiple infection.

The similarity of the distribution curves for single and multiple infection supplements the general finding that bacteria which are simultaneously infected with several virus particles of the same kind react as if only one of the virus particles were effective. We have previously (Delbrück and Luria, 1942) interpreted this phenomenon as self-interference.

The theoretical implications of these results will be discussed in the last paper of this group.

SUMMARY

The burst size distribution for the growth of virus alpha on strain "B" of *Escherichia coli* is determined for single infection. The burst sizes range from below 20 to over 1,000, with a broad maximum around 180.

Comparison with the distribution of bacterial sizes in the same culture shows that the wide distribution of burst sizes cannot be accounted for by variations in the size of the bacteria alone.

Multiple infection with virus alpha gives the same distribution of burst sizes as single infection.

Single and multiple infection with virus delta growing on the same host gives similar distributions, though with higher average burst sizes.

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