GROWTH AND RECOMBINATION IN BACTERIAL VIRUSES[1]

C. LEVINTHAL AND N. VISCONTI
Department of Physics, University of Michigan, Ann Arbor, Michigan,
Department of Genetics, Carnegie Institution of Washington,
Cold Spring Harbor, Long Island, New York

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A THEORY to correlate the various genetic data so far obtained with phage T2 has recently been advanced by VISCONTI and DELBRÜCK (1953). It is known that after infection the phage is transformed into a non-infective form, which has been called vegetative phage. In the model of VISCONTI and DELBRÜCK it is assumed that the vegetative phage particles grow in the bacterial cell and mate pairwise and repeatedly. The pool of vegetative phage has been treated mathematically as a problem of population genetics in which, as time elapses, there is a continual drift towards (i.e., approach to) genetic equilibrium.

When bacteria are broken up at various times after infection no mature particles are found for an interval of time called the eclipse period. Starting at 10 minutes after infection, vegetative phage particles are withdrawn from the pool to become mature infecting particles (DOERMANN 1948). It is assumed that mature phage particles accumulated in the bacterial cell do not return to the vegetative state, which is the only one in which they can grow and mate. The progress of the genetic drift can be measured at the time of lysis and is characterized by the average number of matings between the vegetative phage particles in the pool. The results of many different crosses are explained by assuming that approximately seven rounds of mating have occurred in the pool by the end of the normal latent period.

On the basis of the idea of an approach to equilibrium, it should be expected that the frequency of recombinants would depend on the time of the lysis of mixedly infected bacteria. DOERMANN (1953) has shown that upon lysing very early, a lower frequency of recombinants is obtained than at normal lysis. If recombination values are small (closely linked markers) this difference is barely significant. If the markers are unlinked the difference runs from 34% to 42%.

Some preliminary observations of HERSHEY and CHASE (1951) showed that in lysis inhibited cultures (DOERMANN 1948) the recombination frequency is greatly increased. The aim of the present work is to study this phenomenon of drift toward genetic equilibrium in the progeny phage. On the one hand, closely linked markers should be used in order to avoid differences in the rate of increase of recombinants due to the approach to genetical equilibrium. On

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the other hand, the observations should be extended to periods of time long enough so as to get measurable differences in recombinant frequencies. Recombination between loci h and r_{13} (closely linked) in lysis inhibited cultures satisfies these conditions.

MATERIALS AND METHODS

Strain H of bacteriophage T2 and its host *E. coli H* were used throughout these experiments.

The viral mutations used as markers in our crosses were h (host range), r_{13}, and r_7 (rapidly lysing) (HERSHEY and ROTMAN 1948, 1949). All these markers are in the same linkage group, h being closely linked to r_{13}, less closely to r_7.

The general procedures for the preparation of the stocks and for the assays were those described by ADAMS (1950).

The crosses were made by infecting in buffer with an equal multiplicity of the two parental types, according to the procedure described by VISCONTI and DELBRÜCK (1953).

The method of decompression breakage of the bacteria was the one described by FRAZER (1951) and by LEVINTHAL and FISHER (1952).

The bacteria were counted in a Petroff-Hauser chamber, using dark field phase contrast microscope with a 20-power objective. This objective allowed the use of a thick cover slip, which was necessary in order to get reproducible results with rapid counts.

EXPERIMENTAL RESULTS

Crosses between $h\ r_{13}$ and wild type phages were made with a total multiplicity of infection of ten to fifteen phage particles per bacterium. After adsorption had taken place in buffer, concentrated broth was added to bring the concentration of infected cells to 10^8/ml and the final broth concentration to the normal value. Under these conditions visible clearing of the culture did not occur for several hours. Lysis was presumably inhibited due to the fact that some bacteria liberated phage which was adsorbed on the other bacteria not yet lysed (DOERMANN 1948). Phage assays were made from such cultures at intervals during the period of inhibition. The results are shown in figure 1. Two conclusions can be drawn from this experiment; first the initial rise in phage titer occurs approximately 5 minutes earlier than when the bacteria are diluted immediately after infection. The second conclusion is that the phage titer reaches a constant value after about 25 minutes of growth and remains at this value throughout the period of inhibition. The rise in titer gives a burst size which is comparable to that obtained in the diluted cultures. However, when the frequency of recombinants was measured during the period of inhibition it was found that the phage particles did not remain unchanged; the ratio of recombinants to total number continued to increase up to approximately 90 minutes. Table 1 gives the measurements on the various types of progeny phage obtained after lysis in a normal culture and in a lysis inhibited cul-

ture. Measurements were also made on the recombinant frequencies at various times in an inhibited culture. Figure 2 shows the result obtained in several experiments.

Since it was obvious, from the rise in recombinant frequencies, that the phage particles being scored during the plateau period were not normal free

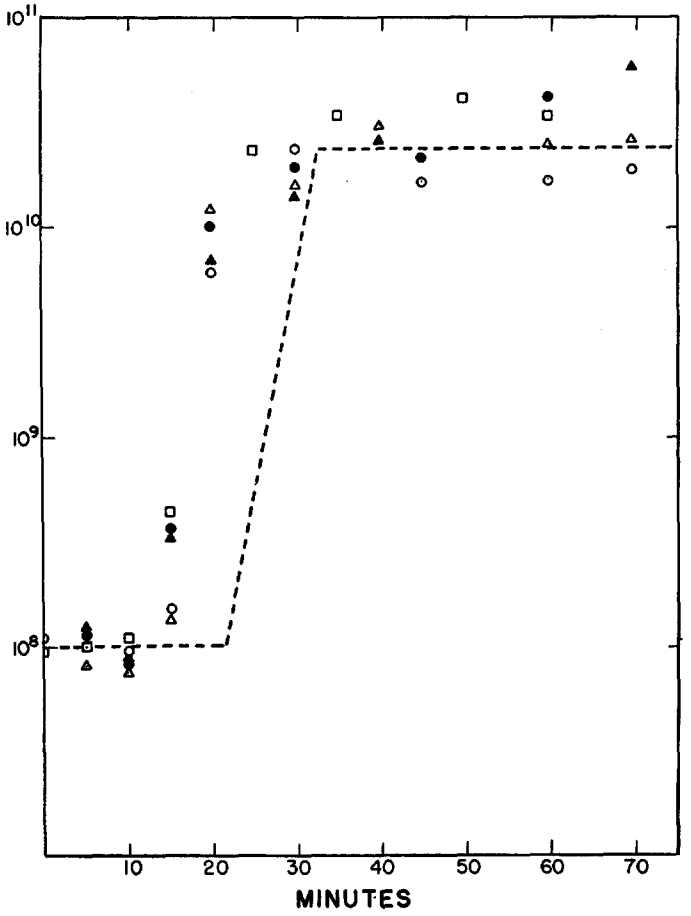

FIGURE 1.—Number of phage particles per milliliter obtained by diluting a lysis-inhibited culture at different times. The different symbols refer to different experiments. The dotted line is a normal one-step growth curve, where the culture is diluted immediately after infection is completed. As stated in the text, the plateau, after 30 minutes, is due to the fact that the increase in the number of phage within the cell is compensated by the loss of cells through lysis.

phage, several experiments were done to investigate their state in the culture. The conclusion reached was that the phage particles which produce plaques between time 20 minutes and the time of visible clearing are not free phage, but are inside the bacteria. These bacteria become extremely fragile and the dilution required for the assay breaks them up and liberates the phage. It was also found that the apparent constancy in the number of phage particles after

TABLE 1

Results of the cross $hr_{13} \times h^+r_{13}^+$ with lysis inhibition and normal lysis. The number of recombinants increases with lysis inhibition. The number of mottled plaques due to the heterozygote phage particles, does not increase (HERSHEY and CHASE 1951).

	h^+r^+	hr	h^+r	hr^+	Mottled plaques	Total	Percent recombinants
Lysis inhibited	568	646	76	90	33	1,413	11.8
Normal lysis	736	702	19	20	31	1,477	2.6

dilution was an accident due to the particular conditions under which the experiment was performed. In reality, phage particles increase in number and are accumulated inside the bacteria, but some of the cells lyse before clearing takes place. The phage particles liberated by these early lysing cells are lost due to readsorption and breakdown (LESLIE, FRENCH and GRAHAM 1950). Under the particular conditions of our culture the number of phage particles broken down just compensated for the growth.

These conclusions are based on the following experimental results:

1. If the culture is centrifuged at a speed sufficient to sediment bacteria but insufficient to sediment phage, most of the phage particles are found in the pellet rather than in the supernatant, as can be seen from table 2.

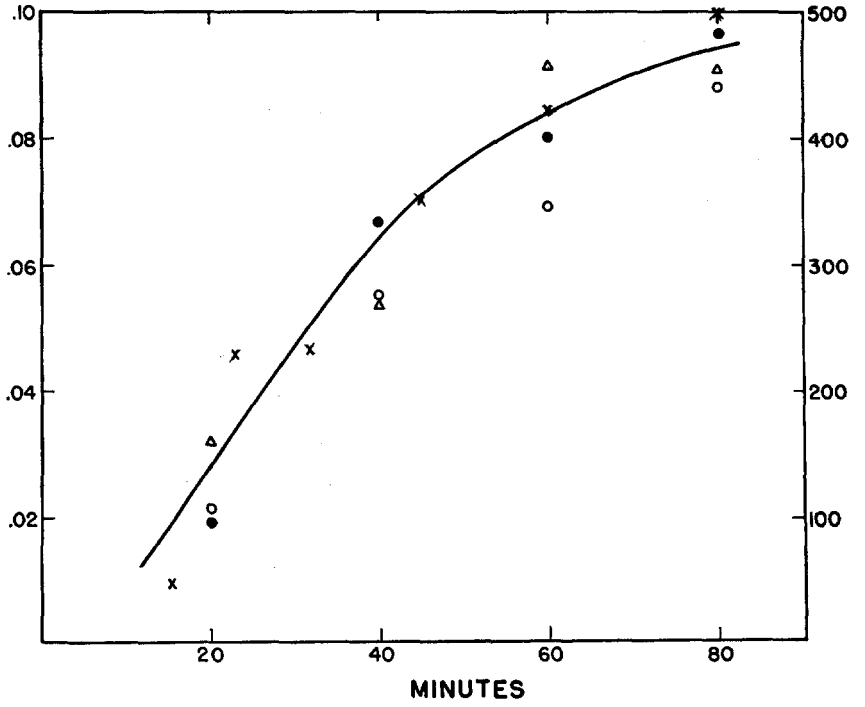

FIGURE 2.—Frequency of recombinants in the cross $h^+r_{13}^+ \times hr_{13}$, plotted against time of dilution from a lysis-inhibited culture. The circles and triangles refer to different experiments. The crosses give the burst size with the scale shown at the right.

TABLE 2

Amount of phage found in the supernatant after centrifuging a lysis-inhibited culture at different intervals of time. In every case the amount is expressed as a fraction of what is recovered, titrating the sample without prior centrifugation.

	Minutes			
	20	40	60	80
Experiment 1	.10	.07	.10	.42
Experiment 2	.15	.12	.18	.36

2. Phage antiserum added to the culture in sufficient concentration to inactivate 90% of the free phage in one minute does not reduce appreciably the titer obtained after dilution, as is shown in table 3.

3. Breaking the bacteria to liberate their phage does not increase the titer obtained after dilution (table 3).

4. If fresh uninfected bacteria are added to the culture at time 30 minutes and then plated at various times on salt-free agar plates, a measure is obtained of the number of free particles in the culture able to kill bacteria. (Plating on salt-free agar prevents the adsorption of the phage to the bacteria on the plate.) From the rate at which the uninfected cells disappear and from a measurement of the adsorption constant of phage to bacteria, we can calculate, approximately, the number of free phage particles. The results show that the number of free phage is approximately 10^8 per ml, which is a hundred-fold less than the amount of phage obtained upon dilution.

5. If phage is added to the culture at time 30 minutes, it disappears at a rate which would be expected from the number of bacteria originally used in the infection.

6. The absorption constant of the freshly liberated phage was measured immediately after dilution. It was found to be the same as that of a normal phage stock.

TABLE 3

Effect of adding antiphage serum to the culture and breaking the cells by explosive decompression. The yields are given as fractions of what is obtained by diluting the culture directly. The data show that the phage obtained is mostly inside the bacteria as it is resistant to phage antiserum. On the other hand, the data show that full yield is obtained by the breakage which occurs at dilution. Previous breakage by decompression does not increase significantly the number of the bacteria which liberate phage.

	60 minutes	90 minutes	After clearing
Treated with phage antiserum for 2 minutes and then diluted	.90	.75	.01
Decompressed and diluted	.95	.90	.80
Diluted	1	1	1

Experiments 1, 2 and 3 demonstrate that the titer obtained before clearing occurs is due to phage particles which are inside the bacteria. The experiments 4, 5 and 6 show that the number of free phage particles in the culture before dilution is small and that these are being continually adsorbed to the cells. In order to check that no further liberation of phage takes place after dilution, the culture was diluted into broth and kept for two hours at 37°C. No appreciable increase was noticed in the phage titer during this time.

Several attempts were made to dilute the infected culture without breaking the bacteria; all these attempts were unsuccessful. The following media were used as diluting fluid: a 1% buffer peptone solution, Difco nutrient broth, a culture of bacteria grown in broth to maximum concentration, buffer at pH 5 and different synthetic media. All these diluting fluids were used at 37°C, at 4°C, and at room temperature.

Because of the failure of this attempt to dilute without breaking the bacteria, there was no biological method available for measuring the number of infected bacteria after 20 minutes of growth. Since it was necessary for the burst size measurements to know this number, counts were made of intact bacteria under the phase contrast microscope. It was separately determined that the microscope counts agreed with the bacterial assays made by a colony-count method, to within about 15% standard deviation. The procedure used in measuring the average burst size during the course of lysis inhibition was to count under the microscope the intact bacteria and to plate a diluted sample in order to titrate the phage. As a precaution, antiserum was added to the undiluted sample to eliminate any free phage, and complete lysis was ensured by the use of decompression breakage. The burst size was calculated by dividing the titer after decompressing by the number of intact bacteria observed under the microscope. Measurements of the burst size made at various times indicated that it increases during the period of lysis inhibition at the same rate as does the frequency of recombinants. This is shown in figure 2. In order to check this point more carefully, measurements were made of burst size and recombinant frequency at two different times in the same experiment. The results are shown in table 4. It can be seen that both frequency of recombinants and burst size increase at the same rate.

A cross was also made between $h\, r_7$ and wild type. In this case the increase in the burst size was the same as in the cross $h\, r_{13} \times$ wild. On the other hand, the frequency of recombinants did not increase by the same amount because genetical equilibrium was being approached. At time 30 minutes the frequency of recombinants was 25%, while at time 90 minutes it increased to 43%. In the same interval of time the burst size increases by a factor of 3, from 200 to 600. In this case the increase in the frequency of recombinants is significantly less than the increase in the burst size.

In order to correlate the increase in the burst size with the increase in frequency of recombinants, it is important to have as few as possible of the infected bacteria lysing early. Since it has been observed that the loss of intact bacteria from lysis is slowed down by infecting at low multiplicities, in all of

TABLE 4

The results of three experiments are shown, in which the percent recombinants and the burst sizes were measured at two different times after infection in a lysis-inhibited culture. Comparison of the last two columns show that the recombinant value is increased by the same factor as the burst size. R_1 and B_1 refer to the earlier time, R_2 and B_2 to the later.

Experiment	Time	Percent recombinants R	Burst size B	R_2/R_1	B_2/B_1
1	32	2.9 ± 0.4	240 ± 30		
	120	8.1 ± 0.8	650 ± 100	2.8 ± .6	2.7 ± .4
2	30	2.2 ± 0.3	220 ± 30		
	90	6.0 ± 0.9	610 ± 90	2.7 ± .5	2.7 ± .4
3	32	4.1 ± 0.6	180 ± 25		
	95	10.5 ± 1.5	600 ± 100	2.5 ± .5	3.2 ± .5
			Average	2.67 ± .3	2.87 ± .5

these experiments the multiplicity was kept close to a value of 10 phage particles per bacterium. As can be seen from the appendix this low multiplicity, because of statistical deviations in the number of phage adsorbed per cell, brings the frequency of recombinants down to 85% of the value that would be obtained if every bacterium had been infected with exactly the same multiplicity of the two parental types. It should be noticed that the values in table 4 fall below the values in figure 2. This discrepancy was accounted for in each case by the low multiplicity of the input.

DISCUSSION

That lysis-inhibited bacteria infected with a mixture of r and r^+ become extremely fragile seems well established in these experiments. The apparent decrease in the latent period under conditions of crowding in the culture is probably another result of this fragility. However, the cause of the fragility has not been determined nor has it been possible to eliminate it by any of the methods tried.

It has been shown in these experiments that when normal lysis is inhibited, there is a period of time which is long compared to the normal latent period, during which phage-infected bacteria can continue to produce and accumulate phage at a constant rate (see also STENT and MAALOE 1952). There is also a linear increase in the recombinant frequency during this extended period so that the number of recombinants increases as the square of the time. After this period of phage increase the cells remain intact for several hours, but there is no further increase in either the total number of phage or in the recombinant frequency. These results support two hypotheses made by VISCONTI and DELBRÜCK (1953). (1) The continual increase of recombinant frequency shows that a cross in the case of phage represents a drift towards genetic equilibrium. Thus a cross between related phages can be explained in terms of a mixed population of vegetative (noninfective) phage particles in which the increase

in recombinant frequency is due to an increase in the average number of matings in the population. (2) The fact that no increase in recombinants is obtained after the growth of mature phage has stopped is to be expected from the hypothesis that only vegetative phage are able to mate or replicate and that particles withdrawn from the pool and matured do not reenter the mating pool.

If we wish to investigate the phenomenon of virus replication there are three basic processes to be considered. First, there is the multiplication of the vegetative noninfective phage particles; second, there is the mating of these particles to produce recombinants; and third, there is the withdrawal of particles from the vegetative pool to form mature infective phage. Under the assumption that mature particles, once formed, do not reenter the pool, the withdrawal process becomes directly observable by measuring the accumulation of infective phage in the cells. The average number of matings among the vegetative particles is also observable since the rate of increase of recombinants among the mature particles is determined by the degree to which genetic equilibrium has been approached in the pool. This can be seen if we consider a short time interval Δt, during which the total number of accumulated phage (N) increases by ΔN. In this same time interval the number of recombinants (R) increases by ΔR. We assume that the maturation process is a random sampling of the pool, and therefore the ratio of recombinants to the total in the pool must equal the ratio of ΔR to ΔN. The recombinant frequency in the pool is now given by $\Delta R/\Delta N$; a measurable quantity.

From the results of the experiments reported in this paper, we can say that both N and R/N increase linearly with time

$$N = Kt; \quad \frac{R}{N} = K't$$

and

$$R = K'tN = \frac{K'}{K} N^2.$$

Therefore, the recombinant frequency in the pool also increases linearly, since

$$\frac{dR}{dN} = \frac{K'}{K} 2N = 2K't$$

Thus we have direct experimental evidence about two of the three processes involved in phage replication. Both the recombinant frequency in the pool and the number of phage particles accumulated by withdrawal and maturation of vegetative particles increase at a constant rate for a period of approximately 60 minutes. From the measured increase in the recombinant frequency, we can calculate that the average rate of matings in the pool must be approximately 1 mating every 2 minutes (VISCONTI and DELBRÜCK 1953).

We have no way of measuring the rate of growth of the particles in the pool, but since the rate of withdrawal is constant, the simplest assumption seems to

be that the pool size is also constant, a condition which would exist if the rate of growth were equal to the rate of withdrawal. The rate is approximately ten particles per minute, which means that ten new vegetative particles would have to be produced per minute. It is tempting to relate the production of new particles to the mating. Since each vegetative phage mates on the average of once every three minutes, an average pool of about 30 particles would be required in order to get one duplication for every mating.

Thus it is possible, with comparatively simple assumptions, to connect growth and recombination in phage, but for a complete demonstration a method should be found to measure, independently from mating and accumulation of mature phage, the rate of growth and the pool size of the vegetative phage.

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SUMMARY

If bacteria are multiply infected with r and r^+ phage, the lysis can be inhibited for several hours by keeping the infected culture concentrated. Cells inhibited by this procedure become extremely fragile and are broken by dilution.

During the first 60 minutes of the inhibition period the average burst size of the cells increases linearly with time as does the recombinant frequency. After 90 minutes growth the increase in recombinants and in burst size levels out, although the cells do not lyse for several hours.

The results are shown to be consistent with the model of VISCONTI and DELBRÜCK (1953) in which the increase in recombinant frequency is due to an increase in the number of rounds of mating in a pool of vegetative, noninfective phage particles. Growth and replication can be correlated by the hypothesis that this pool remains constant at a size of about 30 particles. In this case each particle would mate and duplicate approximately once every two minutes.

APPENDIX

THE EFFECT OF FINITE INPUT IN REDUCING RECOMBINANT FREQUENCY

E. S. LENNOX, C. LEVINTHAL AND F. SMITH

Physics Department and Zoology Department, University of Michigan, Ann Arbor, Michigan

Phage crosses are generally done in mass culture experiments in which it is possible to determine only the average multiplicity of infection of each of the parents. In such experiments there will, of course, be variations about the average since the adsorption is a random process. If the average multiplicity is very high, this variation is small and will make little difference in the expected yield of the cross. On the other hand, if the input ratio of phage to bacteria is low, there will be some cells which receive only one type of parent and thus will produce only a parental type and no recombinants.

For a cross made with equal multiplicity of two parental types, we would have the maximum recombination frequency if each bacterium were infected with exactly the same

multiplicity of each parent. This could in principle be done with single burst experiments or it could be done using very high multiplicity of each. We shall calculate the reduction in this maximum value due to a finite multiplicity of infection in a mass culture experiment.

We consider a single bacterium designated (m, n) infected with m particles of one type, say $++$, and n of the other type, say $--$. Then if we assume random mating, or any other type of random interaction to form recombinants, the number of matings which could lead to recombinants would be proportional to $2mn$. On the other hand, the number of matings of vegetative phage with their own kind which could lead only to parental types would be proportional to m^2+n^2. The recombinant frequency would then be reduced by the factor $4mn/(m+n)^2$ which equals one if m equals n but is less than one otherwise. That this reduction factor is at least approximately correct, has been shown by HERSHEY and ROTMAN (1949) in single burst experiments. This form of the reduction factor is correct even with several rounds of mating, as can be seen from equation 10 of VISCONTI and DELBRÜCK (1953).

Assuming the absorption of phage to be independent random processes, as DULBECCO (1949) has experimentally shown to be essentially correct, we calculate the reduction in the recombinant frequency R_N for those bacteria that have absorbed exactly N phage, m of type 1 and $n = N-m$ of type 2.

$$R_N = 4 \sum_{m=0}^{N} \frac{N!}{m!(N-m)!} \left(\frac{P_1}{P}\right)^m \left(\frac{P_2}{P}\right)^{N-m} \frac{m(N-m)}{N^2}$$

where P_1 = average multiplicity of phage of type 1
P_2 = " " " " " " 2
$P = P_1 + P_2$

Summing, we find

$$R_N = \frac{4 P_1 P_2}{P^2} \left(\frac{N-1}{N}\right)$$

If there is a maximum number ν of phage that can participate in intra-cellular development (DULBECCO 1949), then we take

$$R_N = \frac{4 P_1 P_2}{P^2} \frac{\nu - 1}{\nu} \text{ for } N \geqslant \nu.$$

If we now calculate using the Poisson distribution, the probability P_N that a bacterium absorbs exactly N particles we can find the reduction in recombinant frequency for a mass culture experiment, i.e.,

$$R = \sum_{N=1}^{\infty} P_N R_N$$

$$= \sum_{N=1}^{\nu} \frac{P^N}{N!} e^{-P} \frac{4 P_1 P_2}{P^2} \left(\frac{N-1}{N}\right)$$

$$+ \sum_{N=\nu+1}^{\infty} \frac{P^N}{N!} e^{-P} \frac{4 P_1 P_2}{P^2} \frac{\nu - 1}{\nu}$$

$$= \frac{4 P_1 P_2}{P^2} [F(P) - G(P)]$$

$$\text{where } F(P) = e^{-P} \sum_{N=1}^{\infty} \frac{P^N}{N!} \frac{N-1}{N}$$

$$G(P) = e^{-P} \sum_{N=\nu+1}^{\infty} \frac{P^N}{N!} \left[\frac{N-1}{N} - \frac{\nu-1}{\nu}\right]$$

The sum F(P) can be expressed in terms of tabulated function. We have $F(P) = 1 - e^{-P} + e^{-P}[\ln \gamma + \ln P] - e^{P} \cdot \mathrm{Ei}(P)$, where $\ln\gamma$ the Euler constant is equal 0.5772 and Ei(P) is the exponential integral tabulated in the W.P.A. mathematical tables (New York, 1940).

The function G(P) is the correction to F(P) due to the finite number of phage that can participate in growth. If we take $\nu = 10$ then this correction is only about three percent for P = 20 and about two percent for P = 10. Thus the correction G(P) can generally be neglected as long as the limiting number of participating phage is in the range 10–15 as observed by DULBECCO.

The function F(P) is plotted as a solid line in figure 1. It should be noted that this function rises to its asymptotic value of 1 very slowly and even with high multiplicities of 8 to 10 the recombinant frequency is only 80–90 percent of its maximum value. We can also make a similar calculation for one class of recombinants in a triparental cross. The recombinants (+++) in the cross +−−×−+−×−−+ are reduced due to the fact that each bacterium does not receive an exactly equal input from each of the three parents. This number is reduced by a factor of $\frac{27\, P_1 P_2 P_3}{(P_1+P_2+P_3)^3}$ f(P) where f(P) is a function of the total average multiplicity and is plotted as the dotted line in figure 3.

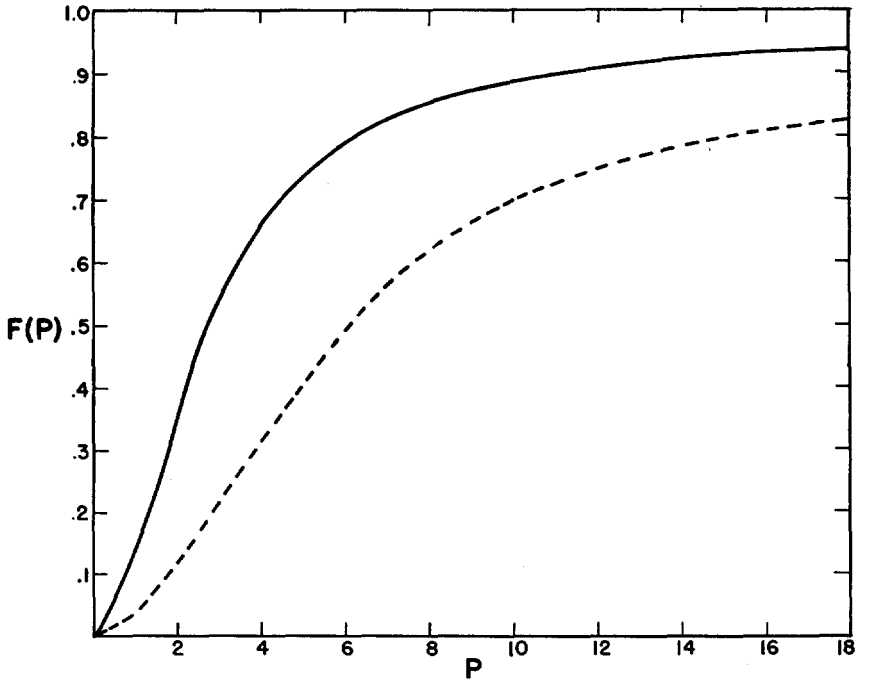

FIGURE 3.—The reduction factor F(P) plotted as a function of the total multiplicity P. The solid curve refers to the bi-parental cross and the dashed curve to the tri-parental cross.

Thus in order to make any comparison between the experimental results of crosses with bacteriophage and models based on a population mixture in single cells, the expected value must first be corrected to account for the variation in the inputs to the individual cells. The correction factor is calculated by multiplying the appropriate function of the total multiplicity, as obtained from figure 3 by $\frac{4 P_1 P_2}{(P_1+P_2)^2}$ for the biparental cross or by $\frac{27 P_1 P_2 P_3}{(P_1 P_2 P_3)^3}$ for the triparental cross, where P_1, P_2 and P_3 are the average ratio of the number of each of the parent viruses to the number of host cells.

LITERATURE CITED

ADAMS, M. M., 1950 Methods of study of bacterial viruses. Methods in Medical Research **2:** 1–73.

DOERMANN, A, H., 1948 Lysis and lysis inhibition with *Escherichia coli* bacteriophage. J. Bact. **55:** 257–276.

1953 The vegetative state in the life cycle of bacteriophage. Cold Spring Harbor Symp. Quant. Biol. **18** (in press)

DULBECCO, R., 1949 On the reliability of the Poisson distribution as a distribution of the number of phage particles infecting individual bacteria in a population. Genetics **34:** 123.

FRASER, D., 1951 Bursting bacteria by release of gas pressure. Nature **167:** 33.

HERSHEY, A. D., and R. ROTMAN, 1948 Linkage among genes controlling inhibition of lysis in a bacterial virus. Proc. Nat. Acad. Sci. **34:** 81–96

1949 Genetic recombination between host-range and plaque-type mutants of bacteriophage in single bacterial cells. Genetics **34:** 44–71.

HERSHEY, A. D., and M. CHASE, 1952 Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36:** 39–56.

LESLEY, S. M., R. C. FRENCH and A. F. GRAHAM, 1950 Breakdown of infecting coliphage by the host cell. Arch. Biochem. **28:** 149–150.

LEVINTHAL, C., and H. FISHER, 1952 The structural development of a bacterial virus. Biochimica et Biophysica Acta **9:** 419.

STENT, G., and O. MAALOE, 1953 Radioactive phosphorus traces. Studies on the reproduction of T4 bacteriophage. II. Kinetics of phosphorus assimilation. Biochemica et Biophysica Acta **10:** 55–69.

VISCONTI, N., and M. DELBRÜCK, 1953 The mechanism of genetic recombination in phage genetics. Genetics **38:** 5–33.