STUDIES ON THE ORIGIN OF BACTERIAL VIRUSES*

I. The Incidence of Phage-Producing Cells in Various B. megatherium Cultures. II. The Effect of Ultraviolet Light on the Incidence of Phage-Producing Cells and of Terramycin-Resistant Cells. III. The Effect of Hydrogen Peroxide on the Incidence of Phage-Producing Cells and of Terramycin-Resistant Cells. IV. Calculation of the Incidence of Phage-Producing Cells

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I. The Incidence of Phage-Producing Cells in Various B. megatherium Cultures

ABSTRACT

Analyses of small samples containing a few cells each show that lysogenic *B. megatherium* produces phage particles in groups of from 10 to 1000 depending on the *megatherium* strain and the culture medium.

These groups probably correspond to the number of particles produced by a single cell. The proportion of such phage-producing cells varies from $<1 \times 10^{-10}$ to about 1×10^{-2} depending on the *megatherium* strain and the culture medium.

If a culture produces two types of phage, the different types usually appear in separate samples. If mixed samples occur, the number of such samples is about what would be expected for the probability that two separate groups would appear in one sample. This result indicates that the appearance of a distinct phage type is the result of a change in the bacterial cell rather than a change in a phage particle, since in the latter case a mixture of the two types would result.

II. The Effect of Ultraviolet Light on the Incidence of Phage-Producing and of Terramycin-Resistant Cells in Various B. megatherium Cultures

ABSTRACT

Low intensity of ultraviolet light increases the proportion of both phage-producing cells and of terramycin-resistant mutants. The increase in phage-producing cells is greater than the increase in terramycin-resistant cells.

High intensities of ultraviolet light cause practically all the cells of some *B. mega-*

* A brief report of this work appeared in the *Proceedings of the National Academy of Sciences*, 1958, **44**, 229.

therium strains to produce phage. The number of terramycin-resistant mutants cannot be determined under these conditions.

The effect of ultraviolet light varies, depending on the *megatherium* strain and the culture medium.

III. The Effect of Hydrogen Peroxide on the Incidence of Phage-Producing and of Terramycin-Resistant Cells in Various B. megatherium Cultures

ABSTRACT

Low concentrations of hydrogen peroxide increase the number of phage-producing cells and of terramycin-resistant cells, concomitantly, from two to five times.

High concentrations of hydrogen peroxide cause almost all the cells of some strains of *megatherium* to produce phage.

IV. Calculation of the Incidence of Phage-Producing Cells

ABSTRACT

The time rate of the appearance of phage particles in normal cultures, or in cultures treated with ultraviolet light or hydrogen peroxide, may be calculated by the same equations which predict the occurrence of terramycin-resistant mutants in *B. megatherium* cultures.

These equations predict that the number of mutants will increase more or less in proportion to the concentration of mutagenic agent, so long as the mutation rate remains very small compared to the growth rate. As the mutation rate approaches the growth rate, there will be a very rapid increase in the proportion of mutants. This explains the striking effect of higher concentrations of mutagenic agents.

In order to calculate the results after exposure to strong ultraviolet light or hydrogen peroxide, it is necessary to assume that the change from normal to phage-producing cell occurs without cell division.

The essential characteristics of bacterial viruses (bacteriophages) are now pretty well established.

They are nucleoproteins (Northrop, 1938; Cohen and Anderson, 1946, Taylor, 1946; Herriott and Barlow, 1954), produced by the bacterial cell (for discussion of the evidence, *cf.* Bordet, 1931; Krueger, 1936; Northrop, 1938; Lwoff, 1954–55, 1957; Herčík, 1954; Raettig, 1955; Northrop and Murphy, 1956; Welsch, 1956), which are capable of transmitting genetic information from one bacterium to another (Freeman, 1951; Stocker, Zinder, and Lederberg, 1953; Lederberg and Edwards, 1953; Kauffman, 1953; Baron, Formal, and Spilman, 1955; Adams and Park, 1956. A change in character of a culture after infection with phage was described by Sonnenschein (1929)). The information is carried by the nucleic acid (Hershey and Chase, 1952). The virus may, therefore, be considered a specialized type of transforming principle (Gratia, 1936 *c*; Lederberg, 1957). Many, if not all, bacterial species produce them. The production of the virus is a genetic property of the host cell (Lederberg and Lederberg, 1953; Wollman, 1953; Fredericq, 1953, 1954). All the cells

of a lysogenic culture are potentially capable of producing virus, but only a few liberate virus particles (Burnet, 1929; Burnet and McKie, 1929; Lwoff and Gutman, 1950). The remaining cells contain no virus (Gratia, 1936), nor any virus antigen (Miller and Goebel, 1954) so that the virus has no continuous existence, and part of the time is indistinguishable from the host cell.

The virus has no metabolism (Bronfenbrenner, 1926; Schüler, 1935; Aji, 1950) and is unable to use energy to synthesize itself; according to the writer's definition (Northrop, 1939 *b*), therefore, it is not living. If it is living, then the reactivation of inactive virus (Krueger and Baldwin, 1933; Krueger and Elberg, 1934; Krueger and Baldwin, 1935; Krueger and Mundell, 1936; Northrop, 1955) is tantamount to the creation of life.

The origin of the virus and its characteristic properties may be accounted for by the assumption that the virus is the result of a mutation of the bacterial cell.[1] After the mutation occurs, the cell produces virus particles instead of more cells. These particles are able to transmit the property of producing virus to other cells. They may be considered, therefore, as "hereditary lethal mutants." The new cell may then occasionally back mutate to give a new lysogenic cell, similar to the original one. This process automatically prunes off any phage-sensitive mutants which may arise, by destroying them or turning them back into phage-resistant lysogenic cells like the original culture.

If this assumption is correct, it would be expected that the proportion of phage-producing cells would be increased by mutagenic agents, and Lwoff and his collaborators (Lwoff, 1954–55) have shown that this is the case.

It would also be expected that other mutants present in the culture would be increased to about the same extent as the phage-producing cells, since mutagenic agents in general increase the proportion of all mutants (Muller, 1954).

The rate of appearance of the virus should be predictable by the same equations which predict the rate of appearance of a known mutant.

In order to test these predictions, the effect of ultraviolet light and of hy-

[1] Since the virus is indistinguishable from the "host" cell, at the time the mutation occurs, it is a matter of convention whether the mutation is ascribed to the virus or the cell (Gratia, 1921; Jacob, 1952; Boyd, 1956; Northrop and Murphy, 1956). The facts are the same as in the appearance of a new enzyme in a cell-enzyme system. This phenomenon is conventionally considered to be the result of a cell mutation; the principle of economy of hypothesis favors a similar convention in respect to a mutation which occurs in a cell-virus system.

There is some direct evidence concerning the origin of the mutation. A single cell produces one type of virus and not a mixture. If the virus mutates as it multiplies, a mixture of virus particles would result, just as in the case of bacterial mutants (Jacobs, 1952). This result was confirmed during the present work. Further, many ways are known to cause a cell to mutate; no ways are known to cause a virus to mutate.

drogen peroxide on the incidence of phage-producing cells and of terramycin-resistant cells (a proven mutation (Northrop, 1957 *a*)) in *B. megatherium* cultures has been determined.

The experiments reported in the present paper show that the predictions are pretty well fulfilled.

I. Incidence of Phage-Producing Cells in B. megatherium Cultures. Determination of the Number of Phage-Producing Cells and of the Number of Phage Particles Produced per Cell

Burnet (1929) and Burnet and McKie (1929) showed that only a few cells of a lysogenic culture produced phage particles. They considered that the rest

TABLE I

Determination of Mutation Rate (λ_P) and Burst Size of Phage-Producing Cells in B. megatherium 899 Cultures at 35°C.

Megatherium 899 in logarithmic growth at 35°, centrifuged, washed, and suspended in YEP; 30 cells/ml. in eighty-six tubes. Shaken at 35° and plated for colonies and phage as noted.

Δt	Colonies/ml.	Cells/ml.	No. of samples	Phage/sample	Ratio Total/negative samples	λ_P
0	8	25	20	0	20/20	
1.5	25	75	60	0	66/60	1.2×10^{-3}
			1	20		
			1	80		
			1	800		
			1	980		
			1	1100		
			1	1180		

of the cells contained an "anlage" for the phage. This result was confirmed and extended by Lwoff (1954–55) and his collaborators, who considered that the non-phage-producing cells contained "prophages."

The ratio of the phage-producing cells to total cells has the dimensions of a mutation frequency rate constant, by definition. This ratio will be called, provisionally, the mutation rate of the phage-producing cells, λ_P, in conformity with the mutation rate of the terramycin-resistant cells, λ_{TR}.

The rate of appearance (mutation rate) of phage-producing cells and also the number of particles produced per cell may be determined from a series of small samples, in the same way that the mutation rate of terramycin-resistant mutants is determined (*cf.* Experimental Procedure).

The results of an experiment of this kind are shown in Table I. Sixty-six samples were plated for phage after 1 hour at 35°. Sixty samples had no phage

particles, two had a few, and four had between 800 and 1200 particles each. There is little doubt, therefore, that in this experiment the phage particles were liberated in lots of about 1000 each. If each lot of phage was the result of a mutation, then there were six mutations from about 5×10^3 cells, corresponding to a mutation frequency rate constant λ of 1.2×10^{-3}. (This is lower than the average value and the burst size is larger.)

All the experiments in which a few samples, only, produced phage particles resulted in counts which formed a group corresponding, no doubt, to the burst size of a single cell. If the fraction of samples containing phage particles is increased by increasing the number of bacteria per sample, it would be expected that some samples would contain two or more bursts, in accordance

TABLE II

Distribution of Phage among Fifteen Samples at 45°C.

Megatherium 899 in YEP. Fifteen tubes containing fifty cells each were shaken at 45° for ½ hour, and the contents of each tube plated for phage.

Phage/sample Class	No. of samples in class		Assumed No. of bursts/sample (500 particles/burst)	Total No. of bursts
	Observed	Calculated		
0 to 300	1	3.6	0	0
300 to 600	8	5.1	1	8
600 to 900	0	0	0	
900 to 1200	5	3.6	2	10
1200 to 1500	1	1.7	3	3
				21

with probability theory. This is sometimes the result, as shown in Table II. In this experiment the number of samples containing one, two, or three bursts is about what would be expected, considering the small number of samples.

The results of a similar experiment carried out with a yellow mutant, Y, resting or in logarithmic growth, are shown in Table III. This mutant produces both C and T phage. In this experiment the mutation rate and also the burst size of the C and T phages are about the same, but are lower when resting bacteria are tested. This agrees with the results obtained with infected cells (Krueger and Mundell, 1938) and is no doubt due to the fact that resting cells are smaller and contain less NA (Price, 1949; Hedén, 1951; Northrop, 1953).

The fractions of samples showing one or two bursts of T or C phage or one or more bursts of both approximate the theoretical expectation.

This result shows clearly that the C and T phage are produced by different cells, as Jacob (1952) has found. This result is to be expected if the virus is the result of a mutation of the "host" cell, but completely unexpected if the mutation occurs to the phage particle. If the mutation occurred as the virus

particles were multiplying in the cell, a mixture of the original virus and the mutant would result. A mixed clone of virus particles would, therefore, appear, just as a mixed clone of bacteria appears after a mutation occurs.

In some experiments in which most of the samples contain phage, the results

TABLE III

Distribution of C and T Phage in Samples Containing Megatherium 899 Yellow Mutant

Mutant 899Y in YEP in logarithmic growth, or resting, centrifuged, washed, and diluted to (1×10^8) cells/ml. 1 ml. in fifteen or thirty tubes, shaken at 35° for 1 hour. Plated for phage.

Culture	Growth rate	Phage	Ratio Total/negative samples	Mutation rate	Average Phage/sample	No. of 1 burst samples		No. of 2 bursts samples		No. of (C + T) samples	
						Observed	Calculated	Observed	Calculated	Observed	Calculated
Logarithmic	2	T	30/13	4×10^{-4}	1000 ± 100	15	10	2	3	10	6.3
Logarithmic	2	C	30/12	4.5×10^{-4}	1100 ± 100	17	10	1	3		
Resting	0.7	T	15/10	2×10^{-4}	500 ± 50	5	3	0	0.5	2	1.2
Resting	0.7	C	15/9	2.5×10^{-4}	600 ± 80	5	4	0	0.7		

TABLE IV

Distribution of Phage among Seventy Samples of Megatherium 899, Each Containing One Hundred Cells after 1 Hour at 35°C.

Class range Plaques/sample	No. of samples in class				
	Observed	Calculated for 15 bursts of 250 each		Calculated for 34 bursts of 2500 each	
			Bursts/sample		Bursts/sample
0 to 200	22	58	0	<1	
200 to 400	9	11	1	<1	
400 to 600	3	1	2	<1	
>600	36	<1	3 or more	<1	
0 to 2000	46	<1		42	0
2000 to 4000	15	<1		21	1
4000 to 6000	8	<1		5	2
>6000	1	<1		1	3 or more

do not agree with simple probability theory, based on a single burst size. An example of this type is shown in Table IV.

The results in this case cannot be accounted for by assuming any one burst size. If a small unit is assumed, there are many more high count samples than would be expected, while a large unit fails to account for the low count samples. The results may be calculated, if it is assumed that there were 15 units of 250 phage particles each and 34 units of 2500 phage particles each.

The high counts may be due to the fact that some cells continue to divide, after the mutation has occurred and phage production has been initiated. This actually happens when *megatherium*-sensitive is infected with C phage in very dilute suspension (Northrop, 1953). Such cells may divide two to three times before lysis occurs and in this case the sister infected cells probably all liberate phage at the same time.

Small bursts would occur if the culture contained some resting cells. Since the culture as a whole was in logarithmic growth, this explanation appears to be unlikely.

The production of phage in bursts may also be demonstrated by determining the phage concentration in a sample containing a few cells, at different time

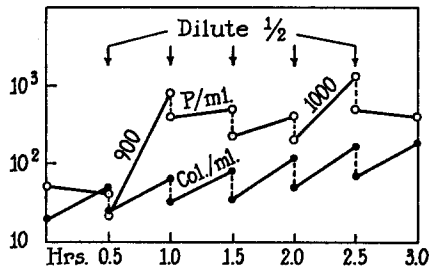

FIG. 1. Increase in the number of phage particles in a culture of *megatherium* 899 containing about 100 cells.

Megatherium 899 in logarithmic growth in YEP centrifuged and washed twice in YEP and diluted to contain about 100 cells/ml. 2 ml. in small test tubes shaken at 35°. Diluted 2/4 every ½ hour and plated for phage and colonies.

intervals. An experiment of this kind is shown in Fig. 1. The culture was diluted one-half every half hour and the colonies per milliliter and phage per milliliter determined. The results show that between 0.5 and 1 hour about 900 phage particles appeared. The number remained the same for an hour and then 1000 more appeared.

Phage-Producing Cells and Terramycin-Resistant Mutants of Various Megatherium 899 Mutants

Since phage production is a genetic property of the host cell, mutant strains of the organism which differ in genetic constitution would be expected to differ, more or less, in the number of phage-producing or terramycin-resistant mutants.

The growth rate, burst size, and the ratio of phage and of terramycin-resistant colonies to bacteria of several *B. megatherium* mutants are shown in Table V. Each mutant has a characteristic value for the phage/cell and terramycin-resistant colony/cell ratios. The ratio of phage/cells $\left(\frac{P}{W}\right)_e$ may be either higher or lower than that of the terramycin-resistant colonies/cells

TABLE V

The Growth Rate, Gelatinase Concentration, Proportion of Phage-Producing and Terramycin-Resistant Cells, and the Mutation Rates of These Cells in Various B. megatherium Cultures at 35°C.

B. megatherium	Culture medium	Growth rate	Equilibrium ratio		Phage type	Mutation rate λ by null fraction		Burst size	Gelatinase units/cells × 10^8
			Terramycin-resistant colonies/cells × 10^8	Phage/cells × 10^8		Terramycin-resistant colonies × 10^8	Phage-producing cells		
899	YEP	1.8	20 to 40	3 × 10^8 1 × 10^2	T C	2.5	3 × 10^{-2}	200 to 500	1 × 10^3
899	Arginine, aspartic acid, 10^{-3} M $MgSO_4$	1.0	30	1 × 10^7	T	2	10 × 10^{-2}	100 to 200	<10
899	Arginine, aspartic acid, 10^{-5} M $MgSO_4$	0.6	50	0.5 × 10^7	T	5	2 × 10^{-2}	$10\frac{1}{2}$ to 20	
899	ASCM	0.4	20	1 × 10^7	T				<10
899 adapted (AC8)	ASCM	1.8		1 × 10^7 1 × 10^7	Small T C + S, etc.				
AC8 adapted to	YEP	1.2	1.5 × 10^3	1 × 10^4	Small T				5 × 10^3
Y	YEP	2.0	20 to 40	1 × 10^6 0.3 × 10^6	T C	4	2.5 × 10^{-4} 2.0 × 10^{-4}	500 to 1000	1 × 10^3
Y1	YEP	2.0	<1						
Y	ASCM	0.7	20	1 × 10^4 3 × 10^2	T C	30	1.5 × 10^{-5} 2.0 × 10^{-5}	2 to 4	<10
SP	YEP	0.5	6 × 10^3	3 × 10^6	T				
KM	YEP	1.8	5 to 500	<1 × 10^{-2}					1 × 10^3

$\left(\frac{TR}{W}\right)_e$. For instance, the ratio of terramycin-resistant mutants per cell in 899 cultures is about 20 × 10^{-8}, the ratio of T phage per cell is about 3, while the ratio of C phage per cell is in the order of 1 × 10^{-6}. The sensitive strain KM and a mutant from lysogenic strain AC8 produce <1 × 10^{-10} phage/cell; a yellow mutant Y1 produces <1 × 10^{-8} terramycin-resistant colonies/cell.

The equilibrium ratio, the mutation rates, and the burst size, also vary with the culture medium. The burst size increases with the rate of growth as in the case with infected cells (Krueger and Mundell, 1938; Hedén, 1951; Northrop, 1953), and there is some indication that the mutation rate also increases with the growth rate, as Zamenhof has found (1945).

The gelatinase concentration is higher in those cultures in which the burst size is high. There is no increase, however, in the gelatinase concentration if the culture is caused to lyse by ultraviolet light or hydrogen peroxide. It is probable, therefore, that changes in the gelatinase concentration are due to

TABLE VI

Terramycin-Resistant Colonies, C Phage, and T Phage from Various Clones of Megatherium 899Y

Ten colonies picked from YEP plate, grown up in YEP to (1×10^8) cells/ml., and plated for phage and terramycin-resistant colonies.

Clone No.	Terramycin-resistant colonies/cell $\times 10^8$	C phage/cell $\times 10^6$	T phage/cell $\times 10^6$
1	80	40	1000
2	35	30	300
3	140	10	100
4	130	2	30
5	20	3	30
6	30	4	70
7	50	6	70
8	100	3	0.1
9	30	10	1
10	10	$<1 \times 10^{-5}$	(Sensitive) $<1 \times 10^{-5}$

changes in the amount of enzyme produced per cell, rather than in the number of cells which produce the enzyme.

The ratios of terramycin-resistant colonies and of T or C phage per bacterium in different clones of the yellow mutant, Y, are shown in Table VI. There is considerable variation in each ratio among the various clones. One clone (No. 10) did not produce any phage, and was sensitive to phage. The variation decreases if the clones are kept in continued logarithmic growth, as in the case of terramycin-resistant mutants (Northrop, 1957 *a*).

II. Effect of Ultraviolet Light on the Incidence of Phage-Producing Cells and Terramycin-Resistant Cells

The effect of ultraviolet light on the growth rate and proportion of phage-producing and terramycin-resistant cells in cultures of 899 is summarized in Table VII. Continuous exposure of 899 in YEP to low intensities of ultraviolet light increases the ratio of phage to cells $\frac{P}{W}$ from about 0.5 to 3, while

TABLE VII

The Effect of Ultraviolet Light on the Growth Rate. Proportion of Phage-Producing and of Terramycin-Resistant Cells, and the Mutation Rates of These Cells in Various B. Megatherium Cultures

The figures for the equilibrium ratios and the mutation rates are the means of six to twelve experiments each. The average deviation of the mean is about ±20 per cent.

B. megatherium	Temperature	Exposure		Growth rate A	Ratio R		Mutation frequency rate			Mutation time rate		Burst size l
		Distance	Time		Terramycin-resistant colonies/cells × 10^8	Phage/cells × 10^8	Terramycin-resistant mutants by null fraction × 10^8	Phage-producing cells: By null fraction	Phage-producing cells: By $\lambda = \frac{R}{2l}$	from $C = 2A\lambda$	from $\frac{M}{M_0} = e^{\lambda(1-C-A\lambda)t}$	
		cm.	*min.*									
899	25	—	—	0.8	15	0.5×10^8		1×10^{-3}	2×10^{-3}	1.6×10^{-3}		150
	25	50 from 2 mm. slit	Continuous	0.8	20	3×10^8			3×10^{-3}	5×10^{-3}		300 to 500
	35	—	—	1.8	20	3×10^8	2.5	3×10^{-3}	5×10^{-3}	10×10^{-3}		200 to 400
	35	10	5 to 10	0.8	20 to 30	20×10^8	5		30×10^{-3}	50×10^{-3}		200 to 400
	35	1	2	0.9		∞ (lysis)					4.4	500 to 1000
899 SP	35	—	—	0.46	3.5×10^3	2×10^6						
	35	5	10		5.6×10^3	5×10^7						
899 Y	35	—	—	1.8	25	1×10^7	6	7×10^{-4}	3×10^{-4}			200 to 400
	35	5	10	0.8	25	1×10^7	7	15×10^{-4}	4×10^{-4}			200 to 400

the ratio of terramycin-resistant colonies to total colonies increases from about 15 to 20 $\times$ 10^{-8}. Part of the increase in the $\frac{P}{W}$ ratio may be due to the fact that the burst size appears to be larger when the cells are exposed to ultraviolet light.

If the culture is exposed for a short time at 35° to stronger ultraviolet light, the mutation rate of the phage-producing cells increases about six times and that of the terramycin-resistant cells about twice.

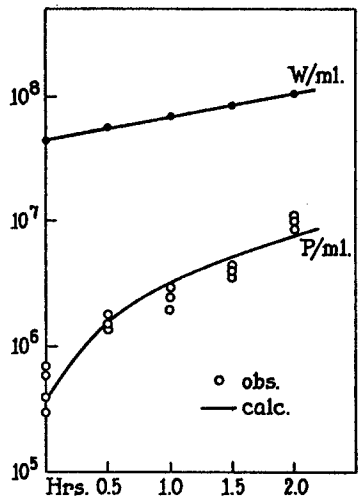

FIG. 2. Phage (P)/milliliter and cells (W)/milliliter in a culture of *megatherium* 899 in YEP at 20°.

Logarithmic growth 899 centrifuged and washed twice in YEP and shaken at 20°. Three phage determinations on each sample.

Results calculated by equations (6) and (9). $l = 50$, $C = 0.8 \times 10^{-8}$, $W_0 = 5 \times 10^7$, $P_0 = 4 \times 10^5$, $A = 0.44$.

If the culture is exposed to strong ultraviolet light so as to cause complete lysis, the mutation time rate C is increased from 10 $\times$ 10^{-8} to 4.4 (Fig. 6) (*i.e.*, phage-producing mutants are formed at the rate of 4.4 per hour) and the mutation frequency rate constant is >0.5. The terramycin-resistant mutants cannot be determined under these conditions, since no colonies are formed.

899Y (the yellow mutant) growing in YEP (at the time these experiments were carried out) was not affected by ultraviolet light. No lysis occurred and there was no increase in the ratio of $\frac{P}{W}$, $\frac{TR}{W}$, or in the corresponding mutation rates.

The rate of appearance of phage particles in a suspension of 899 growing

at 20° is shown in Fig. 2. The results were calculated by equation (6), assuming that the mutation occurs at a rate C, independent of the growth rate A (*cf.* Table I). The value of A was taken from the growth curve of the culture, and W_0, the initial cell concentration, was determined by turbidity.

Fig. 3 shows a similar experiment at 40°. In this experiment, the culture was diluted 1/10 about every hour so as to maintain constant logarithmic growth. The ratio of phage to cells is lower than usual.

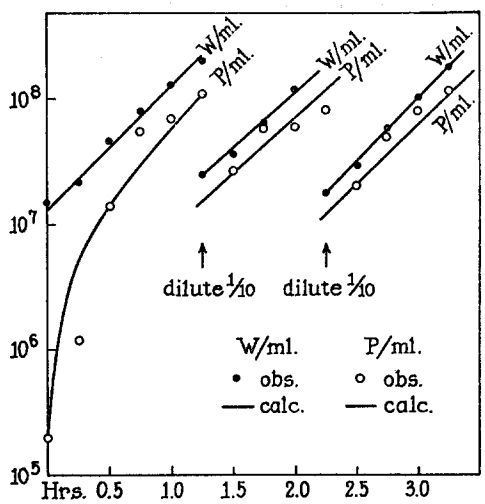

FIG. 3. Changes in cells per milliliter and phage per milliliter in a culture of 899 growing in YEP at 40°C. Experimental procedure same as in Fig. 2. Results calculated by equations (6) and (10). $A = 2.3$, $C = 1.4 \times 10^{-3}$, $P_0 = 2 \times 10^5$, $W_0 = 1.5 \times 10^7$, $l = 700$.

The increase in phage particles in these experiments follows a smooth curve, since a large number of cells are present. If very few cells are present, the increase in phage particles occurs in steps. *Cf.* Fig. 2.

The production of phage by a culture of *megatherium* 899 growing while continuously exposed to weak ultraviolet light is shown in Fig. 4. The mutation time rate constant C is 0.14 in the presence of ultraviolet light instead of 1×10^{-3}. The increase in phage concentration in this experiment is much larger than the average value.

A similar experiment, in which the SP strain was used, is shown in Fig. 5. In this case the ultraviolet light changes the mutation time rate constant of phage-producing cells from 7×10^{-4} to 9×10^{-3}. There was little or no change in the $\frac{TR}{W}$ ratio.

The result of exposure to strong ultraviolet light is shown in Fig. 6. In this case the value of C increases from 1×10^{-3} to 4.4 per hour. The result of this experiment cannot be accounted for if the mutation is assumed to occur as a

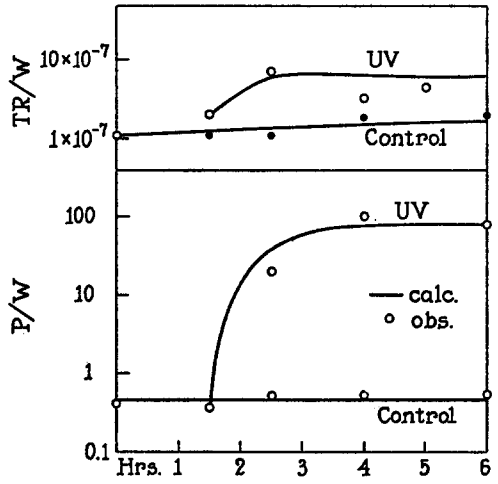

FIG. 4. Growth and phage production of *megatherium* 899 exposed to continuous ultraviolet light.

10 ml. of a suspension of *megatherium* 899 in logarithmic growth in YEP exposed to ultraviolet light at a distance of 50 cm. from a 2 mm. slit in front of General Electric germicidal lamp. Culture stirred with air at 25°C.

Suspension diluted 1/3 about every 1.5 hours.

Cells per milliliter by turbidity. Phage per milliliter by plaque count. Results calculated by equation (7).

In this calculation t = time of exposure − 1.5 hours. This is the time (0.75 to 1 hour) required for the ultraviolet light or its reaction products to make the changes necessary in the bacterial cell. Another 0.5 to 0.75 hour is required for the mutant cell to produce and liberate the phage particles. The mutation itself, therefore, must occur about 45 minutes at 25° before the phage particle appears.

Values of constants used in calculation

Constant	Control	Ultraviolet
l	500	500
C	1×10^{-3}	140×10^{-3}
A	1.0	1.0

result of cell division, since in that case, the rate of disappearance of the wild cells cannot be greater than the rate of growth, before the mutation occurred (Northrop and Kunitz, 1957).

III. Effect of Hydrogen Peroxide on the Incidence of Terramycin-Resistant Cells and Phage-Producing Cells

The results of a series of experiments in which various *B. megatherium* strains were grown in the presence of hydrogen peroxide are shown in Table VIII.

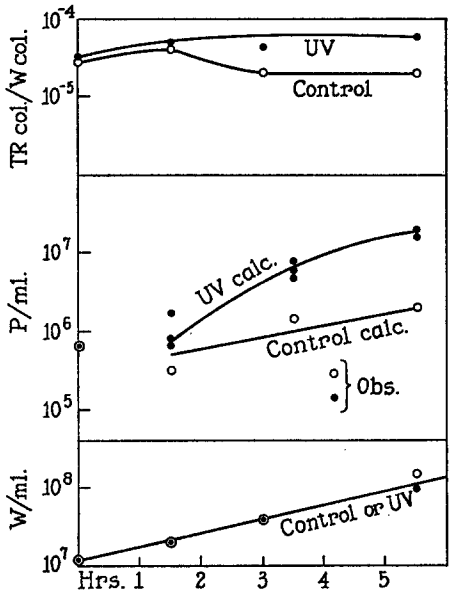

FIG. 5. Effect of ultraviolet light on phage production of *SP megatherium* in YEP at 25°C.

10 ml. YEP containing 1×10^7 cells/ml. in quartz and glass test tubes, placed 50 cm. from 2 mm. slit in front of General Electric germicidal lamp. Suspension stirred by air bubbles. Cells per milliliter by turbidity. Phage per milliliter by plaque count (after toluene). t = elapsed time − 1.5 hours. Results calculated by equation (6).

Constant	Control	Ultraviolet
P_0	5×10^5	5×10^5
l	10	10
C	7×10^{-4}	9×10^{-3}
W_0	2×10^7	2×10^7
A	0.46	0.46

The ratio of terramycin-resistant cells to wild cells and also of phage to wild cells in *megatherium* 899 Y cultures is increased about three times in the presence of (3 to 5) $\times 10^{-5}$ M hydrogen peroxide. This is true in YEP and also in ASCM, although the ratio of $\frac{P}{W}$ is much lower in the latter culture medium.

There is a concomitant increase in the mutation rates, as determined by the null fraction method, showing that the increased ratios are due to the appearance of more mutants, and not simply to a change in the growth rates, or in the number of phage particles formed per cell. Higher concentrations of peroxide give irregular results, frequently lower than the ratio observed at 1×10^{-4} molar. This is the usual result of higher concentrations of mutagens.

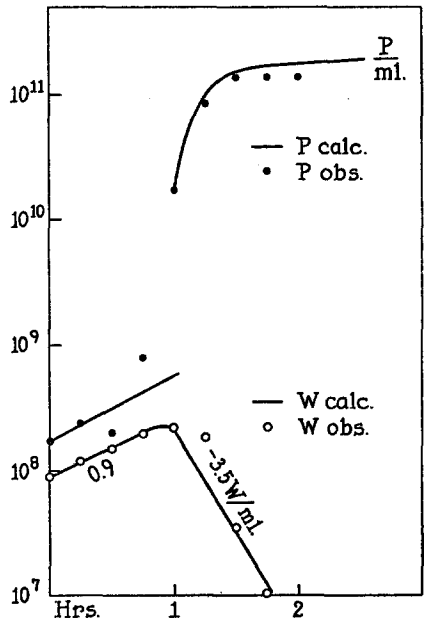

FIG. 6. Effect of strong ultraviolet light on phage (P) and cell concentration (W). *Megatherium* 899 in logarithmic growth in YEP at 35° exposed to General Electric ultraviolet light for 10 minutes at 10 cm. distance and then shaken at 35°.

Results calculated from equation (6). $l = 700$, $C = 4.4$, $A = 0.9$, $W_0 = 2 \times 10^8$, $P_0 = 1.5 \times 10^{10}$, $\lambda = 0.7$, $t =$ (elapsed time $- 1.0$).

The results are calculated *as if* the phage were liberated as soon as the mutation occurred.

The ratios of phage to cells and of terramycin-resistant mutants to cells in cultures of 899 growing in ASCM or aspartic acid–arginine medium containing 1×10^{-4} M/liter hydrogen peroxide also increase concomitantly three or four times.

In YEP, however, there is a sudden increase in the ratio of phage to cells at about 2×10^{-5} M hydrogen peroxide and higher concentrations cause complete lysis.

This confirms Lwoff's (1954–55) observations that lysis is difficult to induce

in synthetic media, in which the ratio of $\frac{P}{W}$ is low. Similar results are reported by Haas and Doudney (1957) in connection with the occurrence of a color mutant of *B. coli*.

The fact that mutagenic agents will cause complete lysis in cultures which

TABLE VIII

The Effect of Hydrogen Peroxide on the Growth Rate, Proportion of Phage-Producing and of Terramycin-Resistant Cells, and the Mutation Rates of These Cells in Various B. Megatherium Cultures

The figures for the equilibrium ratios and the mutation rates are the means of six to twelve experiments each. The average deviation of the mean is about ±20 per cent.

B. megatherium	Culture medium	Hydrogen peroxide	Growth rate A	Equilibrium ratio R		Mutation frequency rate			Burst size *l*
				Terramycin-resistant colonies/cells × 10^8	Phage/cells × 10^8	Terramycin-resistant mutants by null fraction × 10^8	Phage-producing Cells		
							By null fraction	By $\lambda = \frac{R}{2l}$	
		Mol./liter							
Y	YEP	0	1.8	26	4×10^8	4	1.6×10^{-4}	2×10^{-4}	100 to 300
		3×10^{-5}	1.5	70	14×10^8	18	5×10^{-4}	7×10^{-4}	100 to 200
Y	ASCM	0	0.7	20	3×10^8	2	3×10^{-6}	1×10^{-7}	150
		5×10^{-5}		40	10×10^8	6	7×10^{-6}		30
899	YEP	0	1.8	50	3×10^8	2.5	6×10^{-3}	10×10^{-3}	200 to 400
		1×10^{-5}	1.6	80	8×10^8				
		5×10^{-5}			$>1 \times 10^{12}$ (lysis)		>0.5		
899	Arginine, aspartic acid, 10^{-3} M $MgSO_4$	0		6	3×10^8	3	1.6×10^{-2}	1×10^{-2}	100 to 200
		3×10^{-5}		10	8×10^8	5	20×10^{-2}		
899	ASCM	0		7	2.3×10^6				
		1×10^{-4}		30	13×10^6				
KM	YEP	0		5	$<1 \times 10^{-2}$				
		5×10^{-5}		30	$<1 \times 10^{-2}$				

have a high ratio of mutants/cells, but not in cultures with a low ratio is predicted by equation (7) (*cf.* p. 127).

The effect of increasing concentrations of hydrogen peroxide on the ratio of $\frac{P}{W}$, in a culture of 899 in YEP, is shown in Fig. 7.

The solid line is calculated by equation (7) assuming that C, the mutation time rate constant, is proportional to the hydrogen peroxide concentration. This is the usual relation (for lower concentrations of mutagenic agents) and

provides good agreement with the experimental results. In low peroxide concentrations the virus increases in proportion to the hydrogen peroxide concentration, but as the value of C approaches the growth rate, a small increase in the hydrogen peroxide results in an enormous increase in the ratio of virus to cells. This is correctly predicted by the equation.

The rate of appearance of virus in the presence of 5×10^{-5} M hydrogen peroxide is shown in Fig. 8. Under these conditions, practically all the cells produce phage. The rate of decrease of the cells is much faster than the growth rate, and hence, the change to phage production must occur without cell di-

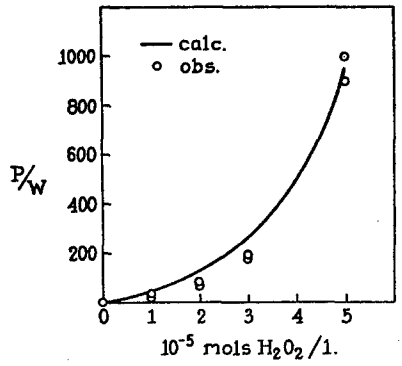

FIG. 7. Ratio of virus particles to cells $\left(\frac{P}{W}\right)$ of *megatherium* 899 in YEP containing various concentrations of hydrogen peroxide after 2 hours' growth at 35°C.

Results calculated from equation (7). $t = 2$, $l = 100$, $C = 0.1 + (3 \times 10^4) \times$ (mols H_2O/liter).

H_2O_2 mols/*liter*	0	1	2	3	5×10^{-5}
A	2	1.6	1	0.7	0.5

vision, since otherwise the rate of decrease of the cells could not be faster than the growth rate, even though every *new* cell produced phage. These results are also predicted quite well by equations (6) and (10). In this case, it is assumed, for ease of calculation, that the mutation occurs at the time the virus appears, but actually the change probably occurs about ½ hour before the virus appears.

899Y1, a mutant clone of Y, which produced no terramycin-resistant mutants for several weeks, could not be induced to produce any in the presence of hydrogen peroxide, although the phage ratio increased.

The sensitive strain KM, on the other hand, which produces no detectable virus, could not be induced to produce virus, although the ratio of terramycin-resistant mutants to cells increased.

In general, if a culture produces less than 1 per cent of the number of mutants

which can be detected, ultraviolet light or hydrogen peroxide would not cause the mutant to appear, since the mutation rates of terramycin-resistant mutants in KM cultures are only increased about five times by either ultraviolet light or hydrogen peroxide.

There are many reports in the older literature of the "production" of bacterial viruses by treatment with various substances, usually mutagens. Similar experiments recently have been reported by Herčík (1956) and Hradečná (1956). Yamafuji (1956) has described the production of polyhedral virus in silkworms by various reagents. Such results are to be expected, if the virus is

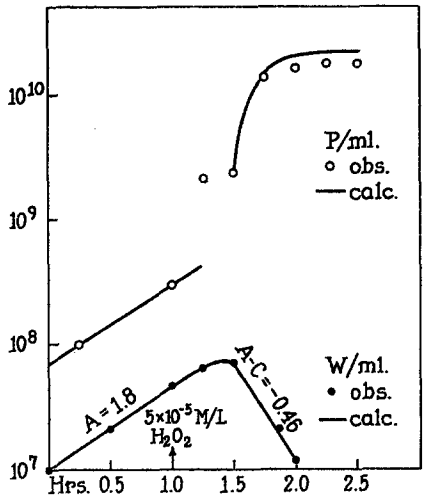

FIG. 8. Change in phage and cell concentration in a culture of 899 growing in YEP at 35°. 5×10^{-5} M H_2O_2/liter added at 1.0 hour. P calculated by equations (6) and (10). $l = 250$, $W_0 = 7 \times 10^7$, $P_0 = 2 \times 10^8$, $C = 6.4$, $A = 1.8$, $W = W_0 e^{(A-C)t}$.

a result of a cell mutation. A virus disease, therefore, may be infectious and also endogenous.

IV. Calculation of the Incidence of Phage-Producing Cells

If the mutants arise as a result of cell division, the number of mutants in a bacterial culture in logarithmic growth is given by

$$M = \frac{2\lambda A W_0 [e^{Bt} - e^{(1-2\lambda)At}]}{B - (1 - 2\lambda)A} + M_0 e^{Bt} \tag{3}$$

(Northrop and Kunitz, 1957)

and the ratio of mutants to wild by

$$\frac{M}{W} = \frac{2\lambda A [e^{[B-(1-2\lambda)A]t} - 1]}{B - (1 - 2\lambda)A} + \frac{M_0 e^{[B-(1-2\lambda)A]t}}{W_0} \tag{4}$$

(Northrop and Kunitz, 1957)

in which A = growth rate of W, λ = mutation frequency rate constant, and B = growth rate of mutants.

In the case of phage-producing mutants, $B = 0$, since the mutants do not grow, and the number of mutants $M = \frac{P}{l}$ in which P is the number of phage particles and l is the burst size; *i.e.*, the particles formed by a single mutant cell.

Substitution of $B = 0$ and $M = \frac{P}{l}$ in (3) and (4) gives

$$P = \frac{2\lambda W_0 l[1 - e^{(1-2\lambda)At}]}{2\lambda - 1} + P_0 \qquad (3 - P)$$

$$\frac{P}{W} = \frac{2\lambda l[e^{(2\lambda-1)At} - 1]}{2\lambda - 1} + \frac{P_0 e^{(2\lambda-1)At}}{W_0} \qquad (4 - P)$$

If $\lambda = 0.5$, the ratio of $\frac{P}{W}$ will remain constant at $\frac{P_0}{W_0}$.

If $\lambda > 0.5$, the wild cells will eventually disappear.

If $\lambda < 0.5$, the ratio $\frac{P}{W}$ will reach a constant value $\frac{P_e}{W_e} = \frac{2\lambda l}{1 - 2\lambda}$.

If $$\lambda \ll 1, \quad \lambda = \frac{P_e}{2lW_e}. \qquad (5)$$

In case the mutation occurs without cell division, the corresponding equations are

$$P = \frac{lCW_0[e^{(A-C)t} - 1]}{A - C} + P_0 \qquad (6)$$

$$\frac{P}{W} = \frac{lC[e^{(C-A)t} - 1]}{C - A} + \frac{P_0 e^{(C-A)t}}{W_0} \qquad (7)$$

If

$$C > A, \quad \frac{P}{W} \to \infty \quad \text{as} \quad t \to \infty$$

If

$$C \ll A, \quad \frac{P}{W} \to \frac{lC}{A} \quad \text{as} \quad t \to \infty \qquad (8)$$

and

$$C = 2\lambda A \qquad (9)$$

also

$$W = W_0 e^{(A-C)t} \qquad (10)$$

Substitution of $2\lambda A$ for C in (6) gives $P = 2\lambda l W_0[e^{At} - 1] + P_0$ which is identical with $(3 - P)$, if $\lambda \ll 1$. Also $\lambda = \frac{P_e}{2lW_e}$, the same as equation (5).

If the mutation rate is low, therefore, the result is the same whether or not the mutants appear only as a result of cell division.

If the mutation rate is significant compared to the growth rate, however, the results predicted by the two mechanisms are very different. As the growth rate approaches 0, for instance, the proportion of mutants will remain constant, if mutants appear as a result of cell division, but will increase rapidly if they are formed without cell division. Also, the velocity constant for the rate of disappearance of the wild cells cannot be greater than A, the growth rate velocity constant, if the mutants appear after cell division, but it can have any value if the mutants are formed without cell division.

In the present experiments it is clear that the change from a normal to a phage-producing cell in the presence of hydrogen peroxide or ultraviolet light occurs without cell division, since the rate of disappearance of the cells is greater than the growth rate. If the mutants appear only as a result of cell division, this could not occur, and the cells would decrease at a rate equal to the growth rate, even though every daughter cell was a mutant.

In the absence of these mutagenic agents, when the mutation rate is very low compared to the growth rate, it is not possible to say whether the mutants appear without cell division, or not, since under these conditions the equations are the same for both conditions. Decreasing the growth rate by decreasing the temperature or changing the culture medium does not result in an increase in the mutation rate of either the terramycin-resistant cells or the phage-producing cells, as might be expected if the mutation occurred without cell division. It may be supposed, however, that changing the conditions also changes the mutation rate in proportion to the growth rate.

The Effect of Increasing the Mutation Time Rate Constant on the Equilibrium Ratio of Mutant to Wild Cells

Equation (7) predicts that if C (the mutation time rate constant) is much smaller than A (the growth rate constant), the equilibrium value of the ratio $\frac{P}{W}$ will increase in proportion as C increases. When C approaches A, however, the value of $\frac{P}{W}$ will increase very rapidly and as soon as $C > A$, the mutants will eventually overgrow the culture. It would be expected, therefore, that mutagenic agents would increase the number of phage-producing cells slowly, at first, but that a critical value would be reached, at which point all the cells would produce phage. This is what happens (*cf.* p. 125). If the mutation rate is small compared to the growth rate, this critical value may not be reached.

Calculation of the Number of Bursts per Sample

The probability P_r that a sample will contain r bursts in n trials, according to Poisson's law of small numbers, is

$$P_r = \frac{(np)^r e^{-np}}{r!}$$

in which n is the number of bursts (trials) and p is the probability of one burst occurring in one sample.

In these experiments, n = the total number of bursts; $p = \frac{1}{s}$ in which s = the number of samples.

The equation, therefore, may be written

$$P_r = \frac{\left(\frac{n}{s}\right)^r e^{-(n/s)}}{r!}$$

The number of tubes containing r bursts is $P_r s$ (Mellor, 1913).

Values of P for various values of $\frac{n}{s}$ and r are given in Pearson (1914) (Table LI). In this table, $x = r$ and $m = \frac{n}{s}$.

Calculation of the Number of Samples Containing Both C and T Phage, Assuming That the Two Types Are Produced Independently

The probability that a sample will contain bursts of both T and C phages will equal the product of the separate probabilities.

$$P_{C+T} = P_T \times P_C$$

in which P_T is the probability that a sample contains 1 or more T bursts, and P_C is the probability that a sample contains 1 or more C bursts.

V. Experimental Procedure

Determination of the Equilibrium Ratio of Phage/Cells and Terramycin-Resistant Colonies/Cells. (Cf. Northrop, 1957 c.)

The culture is maintained in continuous logarithmic growth, by repeated dilution, or in a steady state apparatus (Northrop, 1954), and the number of terramycin-resistant colonies/cell and phage/cell determined at intervals, until a constant ratio is obtained.

The incidence of the terramycin-resistant cells in most cultures is so low that it is necessary to have from (1×10^7) to (1×10^8) cells/sample in order to obtain a reliable count of terramycin-resistant colonies. The phage/cell ratio may be determined from the same sample, but the ratio obtained in this way is not directly com-

parable to the results of the mutation rate experiments, since in the latter experiments the cell concentration is a few hundred/milliliter instead of a few million. The cell concentration affects the number of phage particles liberated per cell, to some extent. The effect of the mutagenic agent also varies with the cell concentration of the suspension. For this reason it is better to determine the phage/cell ratio in separate experiments in which the cell concentration is maintained at <1000/ml.

Determination of the Mutation Rate of Terramycin-Resistant Cells by the Null Fraction Method. (Cf. Lederberg, 1951.)

The determination was made as described previously (Northrop, 1957 *c*).

Determination of the Mutation Rate by the Null Fraction Method and the Burst Size of Phage-Producing Cells

A culture of bacteria in logarithmic growth is centrifuged and washed twice in the culture medium and the cells suspended in 10 ml. culture medium. The bacterial concentration is determined by turbidity and the suspension diluted to contain ten or more cells/ml., depending on the number of phage-producing cells expected. The number of cells present in 1 ml. should be chosen so that from one to six out of ten samples will contain phage after 0.5 to 4 hours, depending on the temperature.

1 ml. of the dilute suspension is placed in twenty or more 15 × 125 mm. test tubes. Two samples are plated for colonies at once and 2 drops of toluene are added to five or more tubes. The remaining tubes are shaken at 35°C. (usually) for 0.5 to 1 hour. Three tubes are then plated for colonies and 2 drops of toluene added to the remaining tubes. All the tubes to which toluene was added are kept at 25° for about an hour. They are then placed in a vacuum desiccator, and kept in the vacuum until the toluene has completely evaporated. This requires 2 to 3 hours.

All the samples are then plated for phage as usual.

The samples to which toluene was added at the beginning must give a negligible number of plaques, compared to the samples in which one or more bursts have occurred, otherwise the number of bursts cannot be determined. Cultures which have a very high mutation rate (>0.01) are not satisfactory, because it is necessary to have a very small number of cells in order to obtain single bursts, and the colony counts are therefore irregular. If very few cells are present per sample, the random appearance of fast growing cells, instead of phage-producing cells, might determine the results.

Calculation of the Mutation Frequency Rate Constant

The mutation frequency rate constant ("mutation rate") is the ratio of mutants to new wild cells, by definition. In the case of phage-producing mutants, there is a complication in determining the number of wild cells owing to the fact that some time elapses between the occurrence of the mutation and the time it can be detected by means of the free virus particles.

The number of cells used to calculate the mutation rate should be the number which were present per sample when the mutation occurred. The exact time of this occurrence is not known, however. Cells which have been exposed to ultraviolet light require about 1 hour to liberate phage particles (less, if covered with toluene or de-

prived of oxygen), while infected sensitive cells require ½ hour at 40°. Cells growing in some culture media cannot be infected, nor caused to undergo massive lysis by ultraviolet light. There is, in addition, a lag in the growth rate of cells which have been centrifuged and washed. This further complicates the determination of the time interval.

In view of the uncertain time required for lysis after the mutation occurs, it seems preferable to use the number of cells present at the time the phage particles were liberated. This method will result in a mutation rate which is too small. If there is a definite number of generations between the time of mutation and the time of virus release (Lwoff and Gutman, 1950), then the mutation rate calculated in this way is greater than the correct value by a constant factor of about 3, but if the time between the two events depends upon some reaction which is independent of cell division, as the temperature effect indicates (Northrop, 1957 *b*), then the correction factor would vary with the conditions of the experiment.

On this basis the mutation frequency rate constant (mutation rate), λ is defined by

$$\frac{2.3 \log \frac{\text{total}}{\text{negative}} \text{ samples}}{\text{Cells per sample at end of experiment}}.$$

Since the phage particles do not multiply, the value of λ may be described by the relation $\lambda = \frac{P}{2lW}$ in which $\frac{P}{W}$ is the ratio of virus to cells at equilibrium and l is the burst size. This method also is somewhat uncertain since the burst size and the ratio of $\frac{P}{W}$ decrease as the cell concentration increases. The value of l can be determined only in very dilute suspensions, while the equilibrium value of $\frac{P}{W}$ is best determined in concentrated suspensions. This fact accounts for the different values of λ, obtained by the two methods.

The Effect of Toluene

Samples from cultures which have a high proportion of phage-producing cells and which liberate a large number of phage particles per cell may be plated directly without the addition of toluene, since the number of colony plaques is small compared to the phage plaques. Samples of cultures, 899 in ASCM, for instance, which have very few phage-producing cells, and which produce only a few particles per cell, must have the cells killed before the sample is plated for phage; otherwise, all samples will contain so many colony plaques that the appearance of the phage plaques is obscured.

The addition of toluene to resting or growing cultures under normal conditions kills the cells but has no effect on the virus (Northrop and Murphy, 1956).

The addition of toluene to samples from cultures which have been exposed to ultraviolet light may have an effect on the phage count.

If toluene is added to such cultures immediately after exposure to ultraviolet light (or if they are allowed to stand at 20 to 25°), the cell concentration decreases slowly without any increase in phage (Fig. 9).

If the cultures are grown up for 25 minutes or more after exposure, at 40°, and then allowed to stand, with or without toluene, complete lysis occurs in 10 to 20 minutes and the phage concentration may increase almost as much as in a culture grown at 40° until lysis occurs. This requires about 1 hour. The cells, therefore, probably contain active virus particles 20 to 30 minutes after exposure to ultraviolet light and these particles are liberated by lack of oxygen. The toluene serves to kill any live cells, and also to prevent the absorption of oxygen.

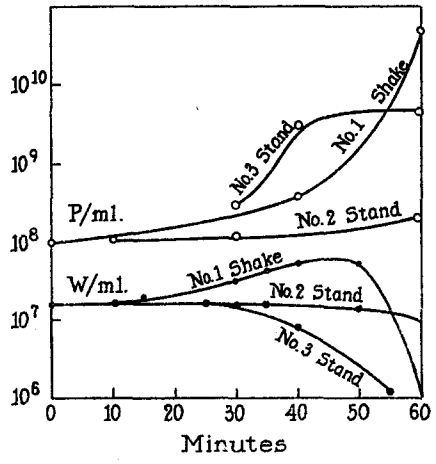

FIG. 9. The effect of standing at 25° on the phage and cell concentration of 899 cultures after exposure to ultraviolet light. 12 ml. of a culture of 899 in YEP in logarithmic growth at 40°, containing 1 × 10^8B/ml., was exposed to ultraviolet light in a quartz test tube for 3 minutes at a distance of 2 cm. The culture was then diluted to 1.7 × 10^7B/ml. and 10 ml. placed in six 22 × 175 mm. culture tubes and shaken at 40°. Two tubes were removed as noted, toluene added to one of them, and both tubes allowed to stand at 25°. B per milliliter by turbidity.

Cultures and Methods of Transfer

B. megatherium 899.—Lysogenic culture descended from de Jong's. This culture has maintained its character, including phage production, for 20 years in this laboratory, when transferred once or twice a week on 2 per cent peptone agar slants (Northrop, 1939 *a*, 1951, 1953; Murphy, 1954; Northrop and Murphy, 1956; Northrop, 1957 *b*, 1957 *c*). Repeated transfer in the steady state apparatus in ASCM results in the appearance of a fast growing mutant which produces a high proportion of various clear plaque phages (Northrop and Murphy, 1956).

Repeated transfer in YEP at 25° in the steady state apparatus results in an increase in T phage and the appearance of about 1 per cent C phage. In this culture the phage-producing cells are at least one-tenth of the total. The culture cannot be used for mutation rate experiments.

Similar changes in *megatherium* 899 cultures have been noted by Gratia (1936 *c*) and by Lwoff, Siminovitch, and Kjeldgaard (1950).

Repeated transfer in the steady state apparatus at 48° results in a culture which produces nearly all C phage.

Transfer in calcium-free medium results in a phage-sensitive strain (Lwoff, Siminovitch, Kjeldgaard, 1950; Clark and Cowles, 1952). If, as seems probable, the phage-sensitive strain is a mutant of the phage-resistant cells, then the phage-sensitive strain will overgrow the culture unless it becomes infected or grows more slowly than the phage-resistant cells.

Changes in the culture, when grown in liquid medium, especially in the steady state apparatus, are to be expected, since under these conditions any mutant which grows as fast or faster than the wild cells will overgrow the culture. A mutant growing on solid medium, on the other hand, cannot overgrow the culture no matter how fast it grows, and the chances are it will be left behind at the first transfer.

Owing to these changes in the culture, the values of the various constants change from time to time and hence, the value of the same constant may be different in experiments done at different times. For comparable results the determination of both terramycin-resistant mutants and phage-producing cells must be carried out at the same time.

Y-Yellow Mutant Isolated from a Colony Plate of 899.—This mutant appeared on a YEP agar plate of 899 colonies July 12, 1956. It produced (3 to 10) $\times 10^5$ T phage and (1 to 3) $\times 10^6$ C + S phage per 10^8 cells and continued to do so indefinitely as long as it was transferred on 2 per cent peptone slants. If transferred repeatedly in YEP it may cease producing terramycin-resistant cells and the number of T and C + S plaques decrease until eventually only a few very small plaques appear.

Continued transfer in ASCM also resulted in the disappearance of the T and C + S plaques and the appearance of many very small plaques. If this culture is then transferred back to YEP, it may produce small C phage only, for some time, but eventually this disappears and a few very small plaques remain.

Colony analysis of such a culture resulted in ten clones which were phage-resistant, but produced no phage which could be detected by the KM strain, two clones which produced small T + C, one which produced a few very small plaques, and two which were sensitive to C phage.

Y1.—Clone from Y which produced no terramycin-resistant mutants for a short time.

AC*8.*—Colony from culture of 899 adapted to ASCM (*cf.* Northrop and Murphy, 1956). This strain originally produced about half C + S and half T phages. After many transfers in YEP, it produced small T only. After standing under oil on a 2 per cent peptone slant, it produced no phage and was sensitive to phage.

Culture Media

Yeast extract peptone (YEP) (Northrop, 1957 *a*).

Ammonium sulfate culture medium (ASCM) (Northrop and Murphy, 1956).

All cultures used in these experiments (unless otherwise stated) were in logarithmic growth at maximum rate. This was obtained as follows. A 10 ml. tube of culture medium was inoculated by loop and allowed to stand at 25° about 18 hours. Under these conditions the culture grows rapidly to about (1×10^7) cells/ml. and then grows slowly due to lack of oxygen. The culture was then diluted 1/10 and the tubes

shaken at the desired temperature until maximum logarithmic growth was obtained. If the maximum growth rate was not obtained before the cell concentration reached the end of the logarithmic growth region, the culture was diluted 1/10 and grown up again at the same temperature.

In some series, the culture was grown in the steady state apparatus at constant maximum logarithmic growth. This method furnished the most reproducible cultures.

Stock Cultures

The stock cultures were maintained in the same culture medium in which the experiments were performed, and transferred by loop every 24 hours to 10 ml. culture medium in 22 × 175 mm. culture tubes. The tubes were kept at 25°. It is important that the cells be grown in the same culture medium as that used in the experiment, since phage production of the cells depends on the medium from which they were taken, as well as that in which they are growing (Northrop, 1951; Lwoff, 1951).

Ultraviolet Light

The culture was transferred to a 2.5 × 15 cm. quartz test tube and placed in front of a General Electric germicidal lamp. The culture was stirred continuously by a stream of air bubbles. Samples were removed as described and shaken at 35°. In the continuous exposure experiments, the lamp was enclosed with a cardboard cover, in which there was a 2 mm. slit.

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CORRECTION

In Vol. **41,** No. 1, September 27, 1957, page 120, in the last line before footnote 1, $\frac{dM_2}{2dn}$ should read $\frac{dM_2}{dt}$.