PHAGE FORMATION IN STAPHYLOCOCCUS MUSCAE CULTURES

X. The Relationship between Virus Synthesis, the Release of Bacterial Ribonucleic Acid, Virus Liberation, and Cellular Lysis

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Previous experiments have indicated that infected cells of strain I of *S. muscae* may release phage under certain conditions without lysing (1). Lwoff and Gutman have recently suggested that the phage is actually liberated while the sample is being diluted for the phage assay, with the cells undergoing lysis at this time (2). It therefore seemed desirable to repeat these experiments in such a way as to rule out this possibility. Earlier experiments have shown that infected cells release ribonucleic acid into the medium shortly after infection and before any virus is liberated (3). Uninfected cells do not release ribonucleic acid into the medium (3).

Recently, various systems have been found in which the virus is adsorbed to the host cell and kills it but no virus is released (4). The evidence indicates that under these latter conditions no *virus material* is synthesized (4). Using these systems, it was possible to study in a more precise manner the relationship between virus synthesis, the release of bacterial ribonucleic acid, and cellular lysis.

The experiments reported in this paper show that infected cells of strain I of *S. muscae* release ribonucleic acid and virus into the medium before cellular lysis begins. Cells of strain III, however, infected under the conditions used to infect strain I, lyse and liberate virus simultaneously, although yielding smaller amounts of phage. Further experiments show that under certain conditions *S. muscae* cells may be infected with one or a few phage particles and be killed without synthesizing any new virus, or releasing ribonucleic acid into the medium, or lysing.

EXPERIMENTAL RESULTS

The Separation of Virus Infection from Bacterial Lysis

Bacteria Infected in Minimal Medium.—Various nutritional systems have recently been described in which amino acids essential for virus synthesis

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have been left out of the medium. Under these conditions the virus is adsorbed to the cell and kills it, but no virus is released (4). Using the aspartic acid–leucine system (4) under conditions in which the majority of the cells were infected with two virus particles, the cells did not lyse, as determined by turbidimetric and microscopic examination, and released no ribonucleic acid into the medium over the 5 hour observation period. The control cells infected in complete medium released 118 γ of ribonucleic acid into the medium 25

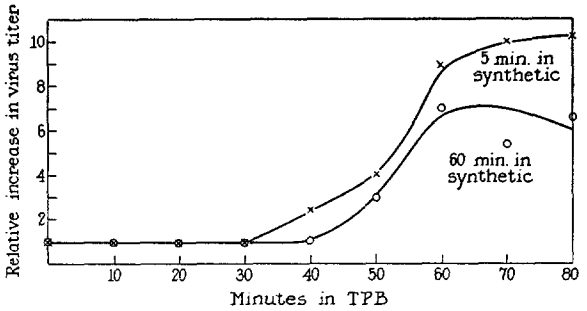

FIG. 1. One-step growth curves of cells infected in the aspartic acid–leucine system for 5 minutes and for 1 hour and then resuspended in tryptose phosphate broth. Two tubes containing 10.0 ml. of minimal medium (10) minus leucine were inoculated with cells which had been grown on the aspartic acid test medium (10) and prepared as described in Methods. Enough cells were added to give 2.0×10^8 cells per ml. After shaking for 3 hours at 36°C., 0.1 ml. of purified virus was added to one tube to give 2×10^9 particles per ml. and an average multiplicity of 3 particles per cell. The two tubes were then shaken for 55 minutes. To the uninfected sample was added the same amount of virus as was added to the first tube. Both tubes were shaken for 5 minutes and centrifuged. Both samples were then suspended in tryptose phosphate broth and a one-step curve carried out as described previously (10). Both these curves show the same time intervals that are observed in normal one-step growth curves of cells suspended in the aspartic acid–leucine system for 3 hours, centrifuged, and resuspended in tryptose phosphate broth and infected.

minutes after infection and started to liberate virus at 35 minutes. The control cells were lysed by 105 minutes.

Results similar to the aspartic acid–leucine system were also obtained with the valine system (4) and the glutamic acid system (4). In all three systems, there was no phage formed as indicated by plaque count. Indeed, there was a decrease in infective centers, beginning about the end of the normal minimum latent period. Since there was no increase in protein or desoxyribonucleic acid in any of these systems (4), it is doubtful whether any virus material was synthesized. Further evidence for this view is the fact that when cells infected for 1 hour in these depleted substrate media were resuspended in complete medium, they showed the same one-step growth curve as is found when cells were in-

fected for only 5 minutes in the minimal medium and resuspended in complete medium. Fig. 1 shows an example of such an experiment.

Bacteria Inhibited by Ultraviolet-Inactivated Phage.—Bacteria were infected with phage inactivated by ultraviolet irradiation as described previously (4). Under these conditions the virus is adsorbed and kills the cells, but no virus is released and the cells do not lyse (4). Such cells did not liberate ribonucleic acid into the medium over the 140 minute observation period, while the control sample infected with the same amount of normal phage liberated 131 γ of RNA into the medium 25 minutes after infection and before any virus was released or before any cellular lysis had taken place. The control cells were lysed within 90 minutes.

Bacteria Infected in the Presence of Iodoacetate and Cyanide.—Bacteria inhibited by such substances as iodoacetate and cyanide form no phage as indicated by an increase in plaque count (4). To see whether such infected cells lyse or release ribonucleic acid into the medium the following experiments were set up.

Three tubes, A, B, C, each containing 10.0 ml. of synthetic medium plus 0.03 ml. of 0.1 M $CaCl_2$ and 5.0 mg. of hydrolyzed casein were inoculated with 2.0×10^8 cells per ml. of strain I prepared as described in Methods. After shaking 120 minutes, at which time the cell count was approximately 2.4×10^8 cells per ml. in all tubes 0.1 ml. of iodoacetate and 0.1 ml. of cyanide were added to tubes A and B, respectively, to give a final concentration of 3×10^{-3} M iodoacetate and 3×10^{-3} M cyanide. After shaking for another 20 minutes, 0.5 ml. of purified phage was added to each of the three tubes to give a final concentration of 1.3×10^9 particles per ml. Over the next 140 minutes tubes A and B, containing the inhibitors, showed no increase or decrease of turbidity within the experimental error of ± 2 per cent. In both tubes, there was a fall in the infective centers beginning about the end of the normal minimum latent period. Tube C began to release phage 35 minutes after the addition of the virus, with the cells being lysed by the end of 80 minutes. The multiplicity of infection in all three tubes was 2. Further experiments, carried out as described previously (3), showed that infected cells inhibited with cyanide and iodoacetate as described above, released no ribonucleic acid into the medium, over the 140 minute observation period, whereas the control cells liberated 181 γ of ribonucleic acid into the medium 25 minutes after infection and before any phage was released and before the cells started to lyse.

The Relationship between Virus Liberation, The Release of Bacterial Ribonucleic Acid, and Cellular Lysis

The Liberation of Virus from Strains I and III.—Fig. 2 shows that there was no decrease in the bacterial count, determined turbidimetrically, of infected cells of strain I, within the experimental error of ± 2 per cent, yet the phage reached its maximum titer. As pointed out previously (1), if such cells are centrifuged out and stained with either methylene blue or the Gram stain, there is no evidence of any cellular debris. However, if the cells are centrifuged out at

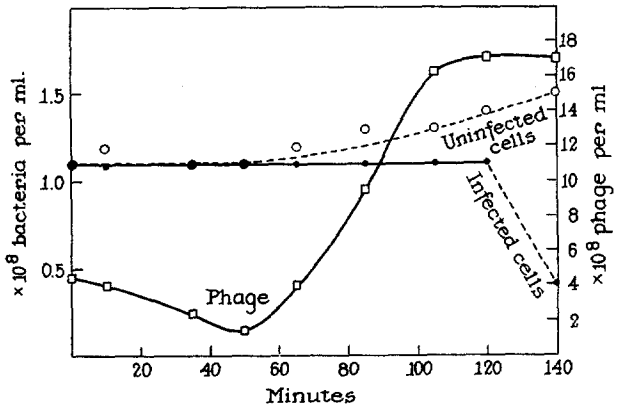

FIG. 2. Release of phage from *S. muscae* cells of strain I. Bacterial cells were washed off 24 hour veal infusion–agar slants and prepared as described under methods. Two 250 ml. Florence flasks, each containing 150 ml. of Fildes' synthetic medium plus 0.3 ml. of 0.1 M $CaCl_2$ plus 30 mg. of acid-hydrolyzed casein, were inoculated with washed cells to give 1×10^8 cells per ml. The flasks were shaken for 60 minutes at 36°C. and then 10.0 ml. of purified phage was added to one flask to give a final phage concentration of 4.5×10^8 particles per ml. The flasks were then put back and shaken at 36°C., with phage and cells determined at varying intervals. All cell counts were determined by pipetting out 12.0 ml. samples from the Florence flasks into 2 × 15 cm. test tubes. Ten colorimeter readings were made on each sample, resetting the colorimeter to zero after each reading. The ten readings were then averaged and this result plotted. This procedure was followed for every determination. The average error of the mean of this turbidity measurement, which was based on the ten readings for a given sample, was ±2 per cent. In assaying the sample for phage, usually two 0.5 ml. samples were removed from the 12.0 ml. sample taken for the turbidity cell count and pipetted into 2 tubes which contained 4.5 ml. of 5 per cent veal infusion. Each sample was then diluted 5 more times, a new pipette being used for each dilution, with a $\frac{1}{10}$ dilution being made each time. 0.5 ml. of the last dilution was then pipetted into 3.5 ml. of tryptose phosphate broth containing 2.0×10^8 cells per ml. 1.0 cc. of tryptose phosphate broth containing 2 per cent agar was pipetted into the tube containing the experimental phage sample, the solution mixed, and then 1.0 ml. of this mixture pipetted onto a Petri dish containing about 10.0 ml. of tryptose phosphate plus 2 per cent agar. After incubation at 36° overnight, the plaques were counted. Two plates were made from each final dilution, the average value of the 2 plates being taken as the phage count for that dilution tube. The two values representing the 4 plates were then averaged and this figure plotted. The phage figures for 10 minutes and 140 minutes represented the average of 10 plates and 8 plates respectively, with all the dilutions being carried out as described above. A viable cell count showed that over 88 per cent of the cells in the flask containing phage were infected. A one-step growth curve carried out with some of these cells gave an average burst size of 13.

a time when the colorimeter indicates lysis, and stained with the Gram stain, a great deal of cellular debris can be seen under the microscope using an oil immersion objective. This indicates that there is not simply cell debris present which gives the same colorimeter reading as the whole cell.

At 35 minutes, at which time 90 per cent of the cells are infected, and at 105 minutes, when the phage count has reached its maximum titer, the cells were centrifuged out. Before centrifuging the 105 minute sample, phage assays were made. Table I shows the cell count of both time samples, and the phage count of the 105 minute sample before and after centrifugation. Within the

TABLE I

Microscopic Bacterial Count and Plaque Count of Cells of Strain I after Infection

At 35 minutes and 105 minutes, as shown in Fig. 1, 10.0 ml. samples were removed from the flask containing the infected cells, pipetted into lusteroid tubes, and these tubes placed in an ice-salt mixture for 2 minutes, as described in Methods. The 105 minute sample was then assayed for phage, 10 plates being made as described in Fig. 2. The samples were then centrifuged and treated as described in Methods. After centrifuging, the supernatant fluids were poured off and a phage assay made on the supernatant fluid of the 105 minute sample, 10 plates of agar being used. The precipitates, containing the cells, were treated as described in Methods and a microscopic cell count made of each sample. 144 small squares were counted for each sample as described in Methods. The total number of cells counted for the 35 minute sample was 3,239, with an average of 23 cells per small square, while a total of 3,538 cells were counted for the 105 minute sample with an average of 24 cells per small square. The phage counts below are based on the 10 plates, with the average figure being given as described in Fig. 1.

Time sample	Total cells counted	Cell count	Phage/ml. before centrifuging	Phage/ml. in supernatant after centrifuging
min.		*ml.*		
35	3239	$9.2 \times 10^7 \pm 0.22$	—	—
105	3538	$9.6 \times 10^7 \pm 0.26$	$16.8 \times 10^8 \pm 8$ per cent	$14.6 \times 10^8 \pm 3$ per cent

experimental error of ± 2.5 per cent there is no decrease in cell count between the two time samples. The 105 minute sample, after centrifugation, showed about 90 per cent of the phage titer found in the uncentrifuged 105 minute sample. The experiment illustrated in Table I and Fig. 2 proves definitely that the virus is released into the synthetic medium under these conditions without cellular lysis and is not released during the dilution of the sample for phage assay as has recently been suggested by Lwoff and Gutman (2). The variations found in this system were reported in an earlier paper (1).

It could be supposed that infected cells multiply, with the virus being liberated from lysing cells. The bacterial count, under these conditions, could remain constant because the number of cells that lyse could be exactly compensated for by the increase in new cells. While such an interpretation cannot

definitely be ruled out, it would seem unlikely, particularly in view of the fact that turbidity measurements, viable cell counts, and, more critically, micro-

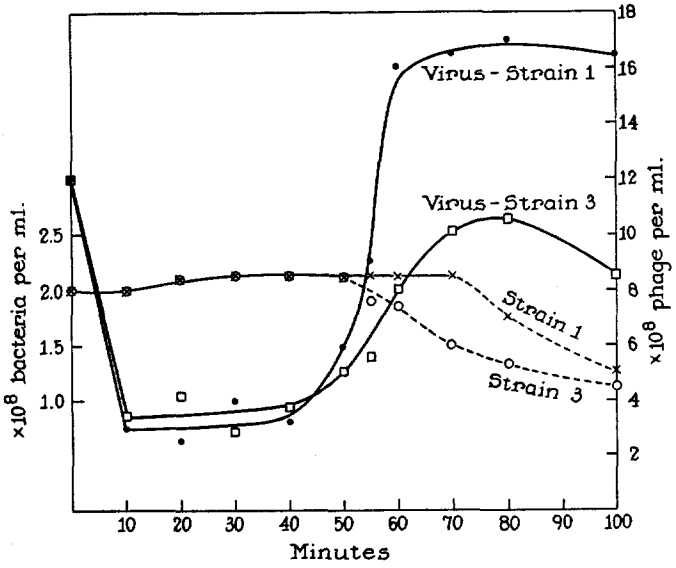

FIG. 3. Comparison of virus liberation and cellular lysis of infected cells of strain I and strain III. Twenty-four hour cultures of strain I and strain III, grown as described in Methods, were washed off their respective slants and prepared as described in Methods. Enough cells were added to 2.0 × 15 cm. test tubes containing 10.5 ml. of synthetic medium (2) to give 2.0×10^8 cells per ml. (determined turbidimetrically). After shaking 45 minutes, 0.5 ml. of purified phage was added to the tube containing cells of strain III to give 1.2×10^9 particles per ml. After 10 minutes, approximately 90 per cent of the cells were infected. Cell counts, determined turbidimetrically, and phage assays were made at the intervals shown in the graph. The tubes containing cells of strain I were inoculated at 60 minutes with 0.5 ml. of the purified phage solution to give 1.2×10^9 particles per ml. After 10 minutes, approximately 90 per cent of the cells were infected. The dotted lines indicate the start of lysis. The cell count, as determined by turbidity measurements, is open to question once the cells start to lyse due to the presence of bacterial debris. The plaque counts are based on the average count of 4 plates, 2 plates being made from dilution series, with each dilution series consisting of seven $\frac{1}{10}$ dilutions. The turbidity measurements are based on 4 readings for each time interval, resetting the colorimeter to zero after each reading. The average of the 4 readings is given in the figure.

scopic examination show that the infected cells cannot multiply. It may be argued that these latter two methods are not critical, since the cells are examined under different conditions than those that exist when the cells are shaken. However, it is felt that the experimental evidence indicates that the infected

cells cannot multiply under the experimental conditions. Bursts from single cells also show that most cells liberate around 10 to 15 particles per cell. There is no evidence from such experiments, that there are a few cells capable of releasing enough phage to account for the increase in titer and still be too few to be detected if they were lysed. Indeed, to reach the observed phage titer, 1×10^8 cells per ml. would have to disintegrate if the phage was released from lysing

TABLE II

Effect of Antiserum on the Virus Released from Strain I and Strain III

Four tubes each containing 10.0 ml. of Fildes' medium plus 5 mg. of hydrolyzed casein and 0.03 ml. of 0.1 M $CaCl_2$ were inoculated with 2×10^8 cells per ml. of strain III. After shaking for 2 hours at 36°C., at which time the cell count was 3.2×10^8 cells per ml., 0.5 ml. of purified phage was added to each tube to give 1.5×10^9 particles per ml. At the end of 60 minutes, when the cells had lysed, the contents of each tube were centrifuged for 10 minutes at $1100 \times g$. The supernatant fluids from each tube were combined and centrifuged at $14{,}000 \times g$ for 2 hours at 10°C. The pellet was dissolved in 5.0 ml. of the above medium and centrifuged for 5 minutes at $1100 \times g$. The supernatant fluid which had a titer of 1.4×10^{10} particles per ml. was used to inoculate 4 tubes prepared as described above. After ten such phage cycles, the resulting phage was used to prepare antiserum to the virus liberated from strain III as described previously (4), and was used as the antigen to test the various antisera. The same method as that outlined above was used to prepare the antiserum and antigen for the virus liberated from strain I, except, of course, strain I was infected rather than strain III. The inactivation of the phage was measured as described previously (4). Two dilution series were made for each phage determination with 2 plates being made from each dilution series. The plaque counts of the 4 plates were averaged and this figure taken for the plaque count of that sample.

Antiserum, diluted 1:100, to phage from	Antigen	Plaque count per ml.	
		Before antiserum	After antiserum
Strain I	Phage from strain I	1.1×10^9	1.2×10^7
	Phage from strain III	1.2×10^9	2.6×10^7
Strain III	Phage from strain I	1.2×10^9	4.1×10^7
	Phage from strain III	1.1×10^9	7.8×10^7

cells. Therefore, even if one assumes that infected cells can multiply, there would have to be the further assumption that infected cells divide faster than uninfected cells, since the uninfected cells would have to double in number if one assumes that the lysing cells were just balanced by the increase in new cells. From Fig. 2, it can be seen that the uninfected cells do not double by the time the phage has reached its maximum titer.

If strain III is infected under experimental conditions similar to those used to infect strain I, there appears to be a strict correlation between cellular lysis, determined turbidimetrically, and virus liberation, even though the virus yield of strain III is lower than that of strain I (Fig. 3).

Experiments were carried out to test the possibility that the virus liberated from strain I was different from that liberated from strain III, since the results obtained with the *E. coli* B system (5, 6) have indicated that different viruses

TABLE III

*The Relationship between the Virus Yield per Cell and the Release of Cellular Ribonucleic Acid into the Medium**

Cells of strain I were grown for 22 hours on veal infusion medium and prepared as described under Methods. 1500 ml. of Fildes' synthetic medium plus 0.03 ml. of 1 M $CaCl_2$ and 500 mg. of hydrolyzed casein was inoculated with 2.0×10^8 cells per ml. This mixture was divided up into three 500 ml. portions and poured into three 1000 ml. Florence flasks. One sample was shaken for 45 minutes, another sample was shaken for 65 minutes, and a third sample was shaken for 180 minutes. After shaking for the appropriate times, the 45 minute sample had 2.0×10^8 cells per ml., the 65 minute sample had 2.0×10^8 cells per ml., and the 180 minute sample had 3.0×10^8 cells per ml. 1.2×10^9 particles per ml. were added to the 45 and 65 minute samples and 1.8×10^9 particles per ml. were added to the 180 minute sample to give an average of 6 virus particles per bacterium. All phage preparations were added in a volume of 5.0 ml. The multiplicity of infection was two in all cases and over 87 per cent of the cells were infected after 10 minutes in all samples. About 6 minutes before the cells started to liberate phage in each sample, 150 ml. of the sample was removed and put into lusteroid tubes which were put into a cracked ice-salt mixture for 2 minutes. The samples were then centrifuged at $2000 \times g$ for 7 minutes at 8°C., the supernatant fluid poured off and 3.0 ml. removed for phage assay, and then 14 ml. of 50 per cent trichloroacetic acid was added to the remaining 147 ml. of the supernatant fluid from each sample. The sample was then put in the ice box overnight and the precipitate analyzed for ribonucleic acid as described previously (3). Phage assay of the supernatant fluids showed that no sample had released phage during the centrifuging within the experimental error of ±7 per cent.

Experiment	Time cells shaken before infection	RNA found in medium from 4.5×10^{10} cells	No. of virus particles liberated per cell	RNA liberated per 10^{11} particles
	min.	*gamma*		
1	45	45	3	33
	65	88	5	39
	180	164	11	33
2	45	54	4	30
	65	88	7	28
	180	242	13	41

* Although there appears to be a rather constant amount of RNA liberated per phage particle, no significance is attached to this at the present time, as this fact is based on too few experiments.

growing in the same host cell may give different lysis results. Antiserum prepared to virus liberated from strain I reacts with the phage liberated from Strain III and *vice versa*. Thus, serologically the virus released from strain I cannot be distinguished from that liberated from strain III (Table II). Furthermore, the virus liberated from strain I can infect and multiply in strain III and *vice*

versa. The plaque type and size of virus released from strain I are the same as the plaque type and size of virus released from strain III. Both viruses need the same amino acids added to the medium in order to grow (4).

The Relationship between Virus Synthesis and the Release of Cellular Ribonucleic Acid and Cellular Lysis.—Earlier results indicated that the release of cellular ribonucleic acid may be an important step leading to cellular lysis (3). However, in measuring the release of ribonucleic acid from infected cells of strains I and III under conditions similar to those described in Fig. 3, both strains started to release ribonucleic acid into the medium at the same time. At 90 minutes strain I had released 104 γ of ribonucleic acid while strain III had liberated only 48 γ of ribonucleic acid into the medium, yet strain III began to lyse at this time whereas strain I did not begin to lyse until 20 minutes later.[1]

Table III shows that the higher the phage yield, the more ribonucleic acid is released into the medium before the cells start to release virus. This is also in accord with the fact that strain I which usually has a larger phage yield than strain III, also liberates more ribonucleic acid into the medium as mentioned above.

DISCUSSION

All the discussion is based on the fact that the two strains of *S. muscae* cells are infected with only one or a few virus particles. Whether the mechanism of lysis is the same under these conditions as it is when the cells are infected with a hundred or more particles is not known. Cells infected with a large number of particles begin to lyse many minutes earlier than cells infected with only one or a few virus particles and form no phage (17). Cells, however, infected with one or a few particles begin to lyse at approximately the same time irrespective of the virus yield (7). This may mean that there are two different lytic mechanisms operating under the two conditions. In this connection, the bacterial lyses of infected cells described in this paper cannot be due to readsorption of liberated phage, since the amount of phage formed, as indicated by plaque count and desoxyribonucleic acid determinations, is far too little to cause "lysis from without" (16).

The results of the experiments reported in this paper may be summarized as follows:—

(*a*) Under certain experimental conditions, the virus is adsorbed to the cell and "kills" it, but no virus material is synthesized as indicated by plaque count, protein and desoxyribonucleic acid determinations, and by one-step growth curves. Such cells do not release ribonucleic acid into the medium and do not lyse.

[1] RNA from infected cells begins to be released into the medium about the time that DNA begins to be synthesized.

(*b*) Phage may be released without cellular lysis from one strain of *S. muscae.* These cells, however, begin to lyse 10 to 20 minutes after virus liberation and also release ribonucleic acid into the medium before the cells start to lyse and before any phage is liberated.

(*c*) In another strain of *S. muscae,* infected under the same conditions as the above strain, virus liberation and cellular lysis occur simultaneously, although the yield of phage is smaller than in the above strain. Such cells release ribonucleic acid into the medium before cellular lysis begins and before any virus is released.

These results show that the release of ribonucleic acid from infected cells is not simply due to the killing of the cell by the virus. The data also show that the killing property of the virus can be separated from its lytic property. Lastly, infected cells have never been found to lyse or release ribonucleic acid into the medium unless virus material is synthesized.

Cohen has stated that the RNA content of *E. coli* B infected with T_2r+ remains constant (8). This investigator did not separate the cells from the medium, but simply added trichloroacetic acid to the whole mixture to give a final concentration of 5 per cent trichloroacetic acid. If any ribonucleic acid or ribonucleoprotein had been released into the medium, it would have been precipitated by the acid and thus the RNA content of the cells would have appeared constant. Murray, Gillen, and Heagy have recently shown that *E. coli* B infected with T_2r+ does lose a great deal of RNA shortly after infection and well before cellular lysis (9).

There are reports in the literature that cells infected with one or a few phage particles lyse and liberate no phage as indicated by an increase in the plaque count (15). The investigators have taken this to mean that cellular lysis can be separated from virus synthesis. However, these experiments do not necessarily mean that lysis under these conditions is not brought about by virus synthesis. It is possible in the above experiments that although no complete phage particles are formed, there is still a synthesis of virus material. This is really the important point, since biochemical data indicate that the formation of the *infective* particle is the last step in a long series of reactions leading to the formation of the complete virus particle (13). A good example of such a case is found in the experiments of Foster (14). She showed that the *E. coli* B cells infected with T_2 in the presence of proflavin, lysed but liberated no infective particles. However, Foster was able to show by the use of the one-step growth curve technique that cells infected in the presence of proflavin were able to carry out the synthesis of virus material, with the inhibitor stopping a late stage in the reproduction of the phage particle. It would seem possible by using different inhibitors together with one-step growth curves, ultraviolet absorption measurements at 2600A, and protein and desoxyribonucleic determinations, to state more precisely whether cellular lysis is related to virus synthesis and if so, what stage in the formation of the complete phage particle leads to cellular lysis.

Experimental Methods

Preparation of Cells and Virus.—Strain I and strain III of *Staphylococcus muscae* were used in all experiments. Strain I was grown on veal infusion agar slants (10), to which was added enough Difco peptone to give a final concentration of 0.5 per cent peptone. Strain III was grown as described previously (10). All the virus used in the experiments waa prepared as described earlier (4).

Determination of Cells and Virus[2].—Cells were determined turbidimetrically as reported earlier (10). Microscopic cell counts were made in a counting chamber, and viable cell counts were made as described previously (10). Virus was determined as described earlier (3). All phage counts were based on the average count of four plates, two plates being made from each dilution series, unless otherwise stated.

Reaction Mixture.—All reaction mixtures were shaken at about 130 oscillations per minute in Florence flasks or in 2.0 × 15 cm. test tubes at 35–36°C.

Aspartic Acid–Leucine Test System.—Cells were prepared as described previously (10).

TABLE IV

Lag Period, Multiplication Time, and Phage Yields of Strains I and III*

Strain	Lag period	Division time	Average burst size of lag phase cells
	min.	*min.*	
I	75–95	120–140	5–15
III	40–70	90–110	3–9

* The lag period is defined as that interval during which the turbidity remains constant within the experimental error of ±2 per cent.

Comments.—In first setting up the "non-lysis" system it is a good idea to set up a series of infected samples starting at 50 minutes after the cells were first suspended in the synthetic medium and infecting samples every 7 minutes from then on up to 80 minutes. This will insure getting at least one tube showing the non-lysis phenomenon. The yield with strain I varied from 5 to 15 particles per average infected cell and for strain III from 3 to 9 virus particles per average infected cell. This yield is lower than that found when the system was first investigated 2 years ago at Princeton, and the reason for the difference in yield between the experiments carried out then and now is not known. That the yield varies from experiment to experiment is not unexpected, nor are the other variations in the system (1), as lag phase cells are known to give much more variable burst sizes, minimum latent periods, and so forth. Table IV shows the lag period, division time, and phage yield of strains I and III. These figures represent the values found when the cells are washed off their agar slants and

[2] The "multiplicity of infection" as used in this paper was obtained from the adsorption data and then calculated from Poisson's equation. In stating that the multiplicity of infection was 3, this means that whereas the bacteria were infected with 1, 2, or more phage particles, the greatest percentage of the total number of bacteria were infected with 3.

suspended in Fildes' synthetic medium containing 0.03 ml. of 0.1 M $CaCl_2$ and 2 mg. of acid-hydrolyzed casein (1) per 10.0 ml. of Fildes' medium. If both strains are infected once the cells begin to multiply, then virus liberation is correlated with cellular lysis (2). The cells usually should be infected about 20 minutes before there is any increase in turbidity. This is generally at 30 minutes for strain III, and 60 minutes for strain I. However, since the lag period varies, several samples should be infected at varying times as described above. An experiment showing the release of virus without cellular lysis is described below.

Example of an Experiment Showing Virus Liberation without Cellular Lysis.—Strain I was grown for 24 hours on veal infusion–peptone agar slants at 36°C., washed three times in Fildes' synthetic medium (11), and suspended in 5.0 ml. of Fildes' synthetic medium. A 250 ml. Florence flask containing 150 ml. of Fildes' synthetic medium plus 0.3 ml. of 0.1 M $CaCl_2$ plus 30 mg. of acid-hydrolyzed casein (1) was inoculated with enough prepared cells to give about 1×10^8 cells per ml. After shaking 60 minutes at 36°C., 10.0 ml. of purified phage prepared as described previously (4) was added to the Florence flask to give a final concentration of 4.5×10^8 particles per ml. The mixture was then shaken at 36°C., and cells and phage determined at the intervals shown in Fig. 2. Cells were determined turbidimetrically using filter 66 as described previously (10). Viable cell counts were made on tryptose phosphate agar as reported previously (10). Phage was determined by Gratia's method (12).

Microscopic cell counts were made in a Spencer bright-line improved Neubauer counting chamber. The following method was used. A 10.0 ml. sample was removed and pipetted into a lusteroid centrifuge tube. The tube was then put in an ice-salt bath for 2 minutes and then centrifuged at 10°C. for 7 minutes at $1100 \times g$. The supernatant fluid was poured off and assayed for phage. The tube was allowed to drain on filter paper, and the precipitate was suspended in 4.0 ml. of 2 per cent formaldehyde and pipetted into a 10.0 ml. volumetric flask. The lusteroid tube was washed out twice more with 2.0 ml. portions of 2 per cent formaldehyde. These washings were also added to the volumetric flask. The volumetric flask was then made up to 10.0 ml. with 2 per cent formaldehyde. The contents of the volumetric flask were then shaken so as to insure thorough mixing, and the contents poured into a 2.0×15 cm. test tube. One-fourth volume of small glass beads was added to the test tube and the tube shaken. After shaking for several minutes, one drop of methylene blue was added to the test tube. A sample of this mixture was then run under a cover slip which was placed on top of the chamber. After waiting 15 minutes, the sample was counted.

Veal Infusion–Peptone Medium.—Veal infusion was prepared according to the Difco directions. 0.5 gm. of Difco peptone was added per 100.0 ml. of veal infusion. Enough agar was added to give a final concentration of 2 per cent.

Reliability of Methods.—The average error of the mean (3) was used to calculate the differences from the mean found in the different determinations for the same time sample.

Determination of Ribonucleic Acid.—Ribonucleic acid in the medium was determined as described previously (3). Although the values are given in terms of free ribonucleic acid, it may be that ribonucleoprotein is released into the medium (Price, unpublished data).

I wish to thank Dr. John H. Northrop for his interest in this work. Most of the experimental work was carried out by Miss Virginia Turri.

SUMMARY

1. Under a variety of conditions in which cells are infected with one or a few virus particles and the host cells are killed, but no infective particles or virus material is formed as indicated by plaque count, one-step growth curve, or protein or desoxyribonucleic determinations, the cells neither lyse nor release ribonucleic acid into the medium.

2. The "killing" effect of *S. muscae* phage is separate from its lytic property.

3. The release of ribonucleic acid into the medium is not simply due to the killing of the cell by the virus, and ribonucleic acid is never found in the medium unless virus material is synthesized.

4. Infected cells of *S. muscae* synthesizing virus release ribonucleic acid into the medium before cellular lysis begins and before any virus is liberated.

5. The higher the phage yield the more ribonucleic acid is released into the medium before any virus is released.

6. Phage may be released from one strain of *Staphylococcus muscae* without cellular lysis, although bacterial lysis begins shortly after the virus is released. In another strain, infected under similar conditions, virus liberation occurs simultaneously with cellular lysis.

7. The viruses liberated from both bacterial strains appear to be the same in so far as they cannot be distinguished by serological tests, have the same plaque type and plaque size, and need the same amino acids added to the medium in order to grow. Furthermore, the virus liberated from one strain can infect and multiply in the other strain and *vice versa*.

8. It is suggested that virus synthesis, in *S. muscae* cells infected with one or a few phage particles, leads to a disturbance of the normal cellular metabolism, resulting in lysis of the host cell.

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