RADIOACTIVE STUDIES OF THE PHOSPHORUS METABOLISM OF T2r+ BACTERIOPHAGE WITH ESCHERICHIA COLI

LOUIS W. LABAW, VERNON M. MOSLEY, AND RALPH W. G. WYCKOFF

Laboratory of Physical Biology, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland

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Studies of the radiophosphorus uptake in systems composed of T2r+ bacteriophage developing on the B strain of Escherichia coli offer one of the most direct approaches that can be made to the metabolic processes involved in the growth of this viruslike agent. Much is already known of the phosphorus content of this bacteriophage, partly from chemical and partly from radioactive analyses. Thus chemical methods have established both the amount of phosphorus and the compounds carrying it (Taylor, 1946). These analyses identify over 90 per cent of a total phosphorus content of about 4.8 per cent as deoxyribose nucleic acid. Radiophosphorus studies have shown that about 70 per cent of the phosphorus in this bacteriophage and in the T6 strain is derived from the growth medium after infection of the host bacteria (Cohen, 1948b; Putnam and Kozloff, 1948).

We have been measuring details of the synthesis of nucleic acid in the E. coli T2r+ bacteriophage complex using radioactive phosphorus as a tracer. Results with E. coli alone have already been published (Labaw, Mosley, and Wyckoff, 1950a); the present paper describes similar data from bacteria that have been diseased with T2 bacteriophage. It has been found in the course of this work that additional information can be obtained when the bacteriophage is grown on E. coli strains the metabolism of which has been considerably reduced (Labaw, Mosley, and Wyckoff, 1950c). Such a reduction in metabolic activity can be accomplished by proper treatment of the bacterial culture with chemicals such as formaldehyde (Labaw, Mosley, and Wyckoff, 1949) and mustard (Herriott and Price, 1948) or with suitable doses of ultraviolet light (Anderson, 1948). For the present experiments ultraviolet irradiation was employed because it has proved especially easy to obtain high yields of bacteriophage from bacteria inactivated by this means. Electron micrographs of such irradiated bacteria demonstrate that the latter succumb to the effects of the ultraviolet exposure only after a couple of hours and that it is during this interval that they can support the growth of bacteriophage (Labaw, Mosley, and Wyckoff, 1950b).

METHODS

The E. coli cultures for these experiments were taken from the same B strain used in the previous study (Labaw, Mosley, and Wyckoff, 1950a) and were maintained as described there. They and the wild type T2r+ bacteriophage used here are the same that have been employed in other work at this labora-
tory. Tryptose broth rather than tryptose phosphate broth has been used as the culture medium to ensure a satisfactory radioactive phosphorus uptake without having a Na$_2$HP$_2$O$_4$ content giving greater than 10$^5$ counts per minute per ml of broth. The procedures for sampling and radioactivity measurements have been the same as in the previous study of E. coli with the following exceptions: (1) the sample volume has been increased from 8 to 50 ml, and (2) samples have been sedimented in the ultracentrifuge at 13,000 rpm for 1 hour during the washing process.

To prepare the ultraviolet-inactivated bacteria, washed saline suspensions of E. coli at a concentration of about 10$^9$ per ml were irradiated in 6-mm-thick layers for 2 minutes at a distance of 1 m from a Hanovia analytic type, 500-watt 115-volt, quartz-mercury lamp. This exposure reduced the number of colony-producing bacteria about 10$^8$ times. Before irradiation, broth was removed by two washings in saline. A similar procedure was followed in the irradiation of bacteriophage; then the concentration of T2 in the irradiated saline suspension and the exposure time were adjusted so that there was about a 10$^7$-fold reduction in the number of developable plaques. The terms "UV coli" and "UV T2" will be used to designate irradiated bacterial and irradiated bacteriophage suspensions prepared in this way.

In these experiments the number of plaque-forming units per ml of bacteriophage was measured by making appropriate dilution platings on 1 per cent tryptose phosphate agar. For this purpose 0.1 ml of the appropriate coli-T2 dilution was mixed on the agar plate with an equal volume of a broth suspension containing about 10$^8$ actively growing coli per ml, and immediately spread on the agar plate, which was then quickly dried in a 43 C oven. Developed plaques were counted after 18 hours' incubation at 37 C.

RESULTS

For the first experiments conducted with unirradiated cultures, unlabeled tryptose broth was inoculated with E. coli from a 1-hour culture and incubated at 37 C until a count of about 10$^8$ coli per ml was reached. The bacteria were then centrifuged from the broth and resuspended in new broth labeled with Na$_2$HP$_2$O$_4$. Half of the resulting volume was immediately inoculated with T2 to give the desired infection ratio. The number of bacteria per ml in the coli half of the culture, the number of plaque-forming units per ml in the coli + T2 half, and the radioactivity in the bacteria and in the coli + T2 sedimented from 50-ml portions of the parallel halves were measured as a function of the time of incubation at 37 C.

The results of a typical experiment are plotted in figure 1, where the open circles refer to the coli infected with T2 and the closed circles to the parallel bacterial culture measured for comparison. The infection ratio of T2 to bacteria was about 8 to 1. The bacteriophage titer after the first 10 minutes (see curve A) may not represent the lowest titer reached in the culture because of the extremely rapid changes in the number of plaque-forming units at that time. The coli + T2 culture became visibly clear after about 4 hours. It is noteworthy
that the number of plaque-forming units became nearly constant only after this interval. Curve C, showing the phosphorus uptake by the coli half of the culture, is similar to those in our previous paper. The phosphorus uptake has greatly diminished by the end of 1 hour, thus reflecting the reduction in average size and the reduced multiplication rate (curve D) reported in the earlier paper. Curve B gives the phosphorus uptake in the culture half containing coli infected with T2. It demonstrates that most of the phosphorus recovered in the coli-T2 complex has been taken up during the first hour even though the num-

**Figure 1.** E. coli infected with T2r+ in tryptose broth labeled with P32 to give a count of 10^6 counts per minute per ml. Curve A = number of T2 plaques per ml in the coli + T2 half of the culture; curve B = phosphorus uptake by the coli + T2 complex from 50 ml of culture; curve C = phosphorus uptake in the bacteria isolated from 50 ml of the coli half of the culture; and curve D = number of colony-forming bacteria in this coli half.

ber of plaque-forming units has tripled after this time, and it suggests that this late rise in titer results from gradual dispersion rather than from continued bacteriophage production. The same amount of phosphorus has been recovered from the coli + T2 sediment before and after visibly complete lysis; this is evidence for the efficiency of the washing process. A more detailed study of the rate of phosphorus uptake during the first 2 hours will be described later (figure 7).

In any investigation using actively growing bacteria, the latter will always utilize an indeterminate amount of P32 before they are dominated by the in-

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fecting bacteriophage. This unwanted initial synthesis is largely suppressed when slowly metabolizing ultraviolet-irradiated E. coli cells are used as host. It was shown some time ago (Anderson, 1948) that bacteriophage can multiply on ultraviolet-irradiated bacteria. We have found (1950c) that this multiplication can be practically as great as with unirradiated bacteria provided the infecting bacteriophage is added promptly after irradiation; we have further found that considerably more phosphorus is taken up by such a UV coli + T2 culture than by a control UV coli culture to which no bacteriophage has been added. This stimulation occurs whether the T2 inoculum is a Berkefeld-filtered lysate or a washed suspension in saline.

Data from a typical experiment using UV coli are shown in figure 2. The phosphorus uptake was measured at half-hourly intervals until after visible lysis occurred at the end of about 4 hours. The bacterial count before irradiation was $1.36 \times 10^4$ colonies per ml; the number surviving irradiation, as shown by colony counts of the UV coli culture, was $2.0 \times 10^2$ per ml, corresponding to nearly a millionfold reduction. After a 1-hour lag, the surviving coli began to multiply logarithmically as shown by curve D. The UVcoli culture took up phosphorus from the labeled tryptose broth for the first 90 minutes (curve C). There is a further rise in this curve after 4 hours, when the survivors have become numerous enough to affect it.

The number of plaque-forming units of bacteriophage in the parallel UV coli + T2 culture is shown (curve A) to become comparable on lysis with the number formed in an unirradiated coli + T2 culture (curve A, figure 1). The shapes of curves A of the two figures do not differ more than do such curves from successive experiments with infected unirradiated coli. The phosphorus utilized by the UV coli + T2 culture, curve B, is 5 times greater than that taken up by the UV coli culture, curve C, in the period before multiplication of survivors begins to interfere. In this as in the first experiment, the phosphorus uptake in the T2-infected cultures is nearly finished in 2 hours.

If it is assumed, as seems probable, that most of the bacteria in this experiment have become infected within 15 minutes after addition of the T2 and that the phosphorus uptake thereafter is involved in bacteriophage synthesis, then at least 95 per cent of the total uptake is involved in this process. There should, accordingly, be a significant correlation between the phosphorus content and the number of T2 plaque-forming units after lysis, provided this phosphorus is contained in mature bacteriophage rather than cellular debris. Comparison between the recoveries of phosphorus and of bacteriophage titer in Berkefeld-filtered cultures point to such a correlation. Thus the titer recovered after filtration of the culture (figure 2) incubated for $5\frac{1}{2}$ hours was 74 per cent of the titer before filtering; the corresponding recovery of radioactivity was 68 per cent. The determination of radiophosphorus in the filtered culture is more apt to be subject to losses during washing than the corresponding determination in the unfiltered culture; even so, correction of the recovery of radioactivity for the loss of titer suggests in this typical case that 92 per cent of the utilized phosphorus is in the mature bacteriophage. A comparison of curves A and B,
figure 2, shows a radioactive content of \(4.52 \times 10^{-8}\) counts per minute per plaque-forming unit in the crude lysate of this experiment. As can be seen from the discussion following figure 5, this can be interpreted as an absolute chemical content.

Figure 2 (left). *E. coli* (1.36 \(\times 10^8\) per ml) irradiated with ultraviolet light infected immediately with T2 in P\(^32\)-labeled tryptose broth (10\(^4\) counts per min per ml). Curve A = T2 plaques per ml in the UV coli + T2 half-culture; curve B = phosphorus uptake by the UV coli + T2 complex from 50 ml; curve C = phosphorus uptake in the bacteria from 50 ml of the UV coli half-culture; curve D = surviving colony-forming bacteria in the UV coli half-culture.

Figure 3 (right). *E. coli* (1.02 \(\times 10^8\) per ml) irradiated with UV and infected with T2 in labeled tryptose broth (10\(^4\) counts per min per ml) after 30 minutes, preliminary incubation in unlabeled broth. Curve notation as for figure 2.

An initial radioactive content can be found in UV coli isolated from 50 ml of broth just before the introduction of bacteriophage (zero time). This reflects a small phosphorus uptake by the UV coli while they were being resuspended in labeled medium following centrifugation from the saline in which they were irradiated. The amount of this zero time correction for the bacterially synthe-
sized radioactive content can easily be reduced by adding the labeled Na₂HPO₄ after resuspension of the coli. In the experiment of figure 2 such a reduction was not necessary because the zero time value was only about 1 per cent of the maximum in curve B.

Since the proliferation of bacteriophage was as great on the irradiated *E. coli* of figure 2 as on actively growing bacteria, it seemed important to see if further reduction in the bacterial metabolism would permit an equally good yield of bacteriophage. In the next experiments this reduction was accomplished by incubating the irradiated bacteria in broth for varying times before adding labeled Na₂HPO₄ and bacteriophage.

Figure 3 summarizes results from such an experiment in which the time of preliminary incubation of the UV coli was 30 minutes. The *E. coli* count before irradiation was $1.02 \times 10^8$ per ml. As in the preceding figures, curve A gives the number of T2 plaques per ml in the UV coli + T2 culture; curve B gives the phosphorus uptake by the UV coli + T2 complex from 50 ml of culture; curve C shows the phosphorus uptake in the bacteria isolated from 50 ml of the parallel UV coli culture; and curve D gives the number of colony-forming bacteria per ml in this half of the irradiated culture to which bacteriophage had not been added. Curve A shows a slight reduction in the total bacteriophage produced. The flat portion in curve A around 1 hour could indicate either a delayed maturation of bacteriophage that is not reflected in the phosphorus utilization or a delayed dispersion of bacteriophage already produced. The radioactive phosphorus associated with each infective bacteriophage unit in the crude lysate this time calculated out to be $4.47 \times 10^{-8}$ counts per minute. This crude lysate was passed through a Berkefeld filter so fine that only 43 per cent of the original titer was recovered in the filtrate. The recovered phosphorus activity was 36 per cent, giving a corrected recovery of activity of 83 per cent. As in the preceding experiment, losses during washing for the determination of phosphorus recovery contribute to this being less than 100 per cent.

When the preliminary incubation of the UV coli in broth is lengthened to 1 hour, there is a significant reduction in the maximum attained bacteriophage titer. Figure 4 summarizes such an experiment in which the bacterial count before irradiation was $0.85 \times 10^4$ per ml. About an hour after adding bacteriophage there was the same kind of plateau in the number of plaque-forming units (curve A) that is evident in figure 3. Neither the subsequent rise in titer nor the phosphorus uptake by the UV coli + T2 culture was as great as in the preceding experiments. There was, however, a somewhat higher number of counts, $5.48 \times 10^{-8}$ per minute per infectious unit, in the crude lysate; in the filtered lysate, 84 per cent of the titer was recovered and 79.5 per cent of the phosphorus activity, giving a corrected phosphorus recovery of 95 per cent.

Figure 5 shows results obtained when the UV coli were incubated for 2 hours before infection. In this case the bacterial count was $7.5 \times 10^7$ per ml before irradiation. There was a further reduction in maximum bacteriophage production, and curve A shows that this is due to there being little rise from the plateau that develops after an hour. A corrected phosphorus recovery could not be
determined because there was not enough bacteriophage produced to give a pellet large enough to wash. The computed number of counts per minute per infectious unit, \(6.70 \times 10^{-8}\), was still higher than it was in preceding experiments. It is possible that these higher values are caused by the production of some bacteriophage which fails to develop into infectious units.

Figure 4 (left). *E. coli* \((0.85 \times 10^8 \text{ per ml})\) irradiated with UV and infected with T2 in labeled tryptose broth \((10^4 \text{ counts per min per ml})\) after a 1-hour preliminary incubation in unlabeled broth. Curve notation as for figure 2.

Figure 5 (right). *E. coli* \((0.75 \times 10^8 \text{ per ml})\) irradiated with UV and infected with T2 in labeled tryptose broth \((10^6 \text{ counts per min per ml})\) after a 2-hour preliminary incubation in unlabeled broth. Curve notation as for figure 2.

The relationship between the phosphorus content in the coli + T2 lysate and the T2 plaque-forming bacteriophage units is summarized, together with other data, in table 1. The weights of phosphorus in column 5 were computed as follows from the \(\text{P}^{32}\) counts using the results of a typical chemical analysis, which showed that the dehydrated broth contained 0.64 per cent phosphorus, of which 0.16 per cent was organic in combination. The culture medium used
contained 20 g of the dehydrated broth per liter. The radioactive inorganic phosphorus concentration was kept at $10^4$ counts per minute per ml, or was normalized to this value for presentation of the data. Since only the inorganic phosphorus is labeled by the added $^{32}$P, the conversion factor becomes 

$$\frac{0.64 - 0.16}{20} \times 10^{-8} \text{ g P/ml} = 9.6 \times 10^{-10} \text{ g phosphorus per count per minute.}$$

An average of the most reliable determinations of phosphorus content per infectious unit from the preceding experiments (figures 2, 3, 4), together with one additional result (figure 6), is $4.83 \times 10^{-17}$ g P per bacteriophage particle. This falls within limits set by results of chemical analyses of purified bacteriophage.

**TABLE 1**

Correlation between phosphorus content in the coli + T2 lysate and the number of T2 plaque-forming units, together with recoveries of phosphorus and titer in filtered lysate

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>CRUDE LYSGATE</th>
<th>FILTERED LYSGATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lyssate Titer</td>
<td>Phosphorus per plaque-forming unit g/g</td>
</tr>
<tr>
<td></td>
<td>TYPE T2</td>
<td>P4 in coli + T2</td>
</tr>
<tr>
<td>-Coli + T2 (figure 1)</td>
<td>2.30 X 10**14</td>
<td>6.08 X 10**2</td>
</tr>
<tr>
<td>-Coli + T2 (figure 7)</td>
<td>1.22</td>
<td>8.00</td>
</tr>
<tr>
<td>UV col (0) + T2 (figure 6)</td>
<td>1.22</td>
<td>8.55</td>
</tr>
<tr>
<td>UV col (0) + T2 (figure 2)</td>
<td>2.44</td>
<td>5.02</td>
</tr>
<tr>
<td>UV col (1) + T2 (figure 5)</td>
<td>1.82</td>
<td>4.07</td>
</tr>
<tr>
<td>UV col (1) + T2 (figure 4)</td>
<td>0.73</td>
<td>2.00</td>
</tr>
<tr>
<td>UV col (2) + T2 (figure 6)</td>
<td>0.14</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*This number in parentheses represents the time of incubation in hours after irradiation before infection with bacteriophage.*

phage. Thus Taylor (1946) and Hook, Beard, Taylor, Sharp, and Beard (1946) found an average nitrogen content per infective T2 unit grown in broth of $1.40 \times 10^{-16}$ g. With a phosphorus to nitrogen ratio of 0.358 (Taylor, 1946), the phosphorus content becomes $5.02 \times 10^{-17}$ g P per infective unit, as averaged from a spread of values between $3.3 \times 10^{-17}$ to $8.2 \times 10^{-17}$ g. If it is assumed that our $4.83 \times 10^{-17}$ g P per infective unit utilized from the medium is only 70 per cent of the total phosphorus in the bacteriophage (Cohen, 1948b; Putnam and Kosloff, 1948), this total, $6.9 \times 10^{-17}$ g per infective unit, still is compatible with the chemical values.

There is a very interesting correlation shown in table 2 between the total phosphorus uptake by irradiated coli cultures infected with T2 and the phosphorus synthesized into deoxyribose nucleic acid (DNA) by this number of unirradiated bacteria during the average interval between cell divisions (one
generation lifetime). The figures in column 3 of table 2 give the P32 uptake used for DNA synthesis by 50 times the number of actively growing bacteria shown in column 2 during one generation lifetime. This was computed from experimental data in our previous paper showing that the average P32 uptake per colon bacillus actively growing in tryptose broth was $4.0 \times 10^{-3}$ counts per minute per organism. Of this, 19 per cent presumably was synthesized into DNA1 (Taylor, 1946) during a mean generation lifetime of 23 minutes. The numerical values in column 4 give the total P32 utilized by the UV coli + T2 minus (as initial correction) the P32 taken up by the parallel UV coli culture during the first 10 minutes. This is, of course, only an approximate correction for the P32 used before all bacteria were infected, but even in its entirety it is less than 10 per cent of the total P32 taken up. The comparison of columns 3 and 4 given in column 5 suggests that

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>COLI COUNT BEFORE IRRADIATION, COLI PER ML</th>
<th>P32 UPTAKE FOR DNA SYNTHESIZED INTO DNA BY PHOSPHORUS IN 1 GENERATION, C/MIN IN 50 ML</th>
<th>TOTAL P32 UPTAKE IN UV COLI + T2 CORRECTED, C/MIN IN 50 ML</th>
<th>% COLUMN 4 OF COLUMN 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV coli (0) + T2</td>
<td>$0.82 \times 10^4$</td>
<td>$3.11 \times 10^4$</td>
<td>$3.15 \times 10^4$</td>
<td>101</td>
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<tr>
<td>(figure 6)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>UV coli (0) + T2</td>
<td>1.36</td>
<td>5.15</td>
<td>5.32</td>
<td>103</td>
</tr>
<tr>
<td>(figure 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV coli (1) + T2</td>
<td>1.02</td>
<td>3.87</td>
<td>3.91</td>
<td>101</td>
</tr>
<tr>
<td>(figure 3)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>UV coli (1) + T2</td>
<td>0.85</td>
<td>3.22</td>
<td>1.93</td>
<td>60</td>
</tr>
<tr>
<td>(figure 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV coli (2) + T2</td>
<td>0.75</td>
<td>2.85</td>
<td>0.44</td>
<td>15</td>
</tr>
</tbody>
</table>

* The P32 taken up during the first 10 minutes by the UV coli culture was subtracted as a correction from the total P32 taken up by the UV coli + T2 culture.

The phosphorus utilized for the production of bacteriophage does not exceed that synthesized into DNA during one generation of the actively growing bacteria. It approximately equals this amount until the UV coli culture has been incubated for 1 hour or longer, and cannot therefore support the usual bacteriophage proliferation (last two rows of column 5).

The next two experiments were designed to furnish more detailed knowledge of the phosphorus uptake during the first 2 hours after the addition of bacteriophage. In the experiment of figure 6 the UV coli were inoculated with bacteriophage promptly after irradiation (conditions like those for figure 2). Figure 6a contains the usual curves of phosphorus uptake and bacteriophage titer. The number of colony-forming bacteria before irradiation was $0.82 \times 10^4$. Curve A has a plateau around 1 hour similar to that of curve A of figure 3 where the UV coli were incubated one-half hour before infection; the maximum titer, finally

1 Taylor's analysis was made on an 8-hour E. coli culture.
attained about 5 hours after infection, was $1.22 \times 10^{10}$ T2 plaques per ml (see table 1). The data of curves B and C for the first 90 minutes are plotted on a linear scale in figure 6b. This brings out their divergence and also makes it easy to measure graphically and at frequent intervals the slopes of the smooth curves drawn through the experimental points. These slopes taken at 2½-minute intervals and expressed as the average uptake (in counts per minute per minute) give the small circle of figure 6c. The smooth curves through these small circles represent the rates of uptake of phosphorus. They bring out strikingly the stimulation of phosphorus uptake that follows addition of bacteriophage. The

![Graph](http://jb.asm.org/)

*Figure 6. E. coli (0.82 \times 10^8 per ml) irradiated with UV and promptly infected with T2 in labeled broth (10^4 counts per min per ml) (same conditions as for figure 2). Graph a shows the uptake of phosphorus over the first 2 hours; graph b shows this same uptake on a linear plot; and graph c shows the rate of uptake averaged over every 2.5 minute interval from graph b.*

rate of phosphorus uptake in the UV coli culture alone, curve C, decreases steadily in an approximately exponential fashion, whereas in the parallel culture infected with T2 the rate rises sharply about 7 minutes after infection and attains a maximum rate in about 17 minutes. This maximum rate occurs within the latent period of 20 minutes found from one-step growth curves made under similar cultural conditions. After 90 minutes the uptake rate curves B and C become indistinguishable within the accuracy of the uptake curves of figure 6b.

At its maximum rate phosphorus is utilized (figure 6c) by the UV coli + T2 culture 9 times as fast as by the parallel UV coli culture. It is noteworthy that this maximum of 1,350 counts per minute taken up per minute by the UV coli infected with T2 in 50 ml of the culture is about 19 per cent of 7,100 counts per minute per minute, the rate of phosphorus uptake by an equal number of ac-
tively growing colon bacilli. Since, as already mentioned, chemical analysis shows 19 per cent of the total phosphorus of E. coli in the DNA fraction (Taylor, 1946) and since other analyses show synthesis of DNA but not of ribose nucleic acid by T2-infected cultures (Cohen, 1948a), the rate of phosphorus uptake during bacteriophage production seemingly approaches a limit set by the synthesizing mechanism for DNA in the actively growing E. coli. This result makes it conceivable that the bacterial DNA-synthesizing mechanism is undamaged by irradiation but lies dormant in the nonproliferating UV coli until activated by the need for bacteriophage nucleic acid.

Figure 7. Nonirradiated E. coli infected with T2 in labeled tryptose broth (10^8 counts per min per ml). Graph a is similar to figure 1 with the addition of curve E giving the average P^{32} content per bacterium grown after the introduction of the radioactivity; graph b shows the uptake of phosphorus on a linear plot; and graph c shows the uptake from graph b averaged over every 2.5-minute interval.

A more detailed study of the early stages of an experiment using unirradiated bacteria is summarized in figure 7, which therefore is to be compared with figure 1. Besides the usual curves A, B, C, and D, curve E has been added to figure 7a. This curve E gives the average phosphorus content per bacterium formed after the introduction of the labeling phosphorus (Labaw, Mosley, and Wyckoff, 1950a). As in figure 6, the phosphorus uptake curves B and C have been re-

\[2\] Derived (Labaw, Mosley, and Wyckoff, 1950a) by dividing the total uptake during one generation of \(8.2 \times 10^7\) coli per ml \(\times\) 50 ml in tryptose broth by the mean generation lifetime of 23 minutes.
plotted on a linear scale (figure 7b) for determination of the rates of phosphorus uptake per minute by the bacteria (curve C) and by the E. coli + T2 (curve B) at 2.5-minute intervals. These rate curves are plotted in figure 7c together with a repetition of curve B of figure 6c.

The rates of phosphorus uptake for bacteria with and without the addition of bacteriophage are reversed compared to those of figure 6, which dealt with irradiated organisms. The early rise in the rate of phosphorus uptake for the actively growing coli (curve C of figure 7c) probably expresses their accelerated growth when placed in fresh (labeled) broth after sedimentation from the 1-hr parent culture. This rate curve C is much more sensitive to changes in phosphorus uptake than is curve E of figure 7a. Its relatively broad maximum (figure 7c) corresponds to the rate of phosphorus utilization by actively growing E. coli before reduction in mean bacterial size and nonlogarithmic multiplication have caused the decreasing uptake rate apparent after 15 minutes; if the experiment had been started with a lower bacterial count (10^7 per ml for example), this maximum would have been broader.

The maximum rate of phosphorus uptake for E. coli + T2 appears to be about 38 per cent of the maximum for uninfected E. coli (curve B, figure 7c). This does not, however, mean that the maximum rate of synthesis of DNA in coli + T2 is necessarily twice that in healthy E. coli. The peak in the rate curve B could arise from a continuation of the preinfection synthesis of DNA by the bacteria combined with a gradual reduction of the other phosphorus utilizations. Thus, if it were assumed that the DNA synthesis by actively growing E. coli is not interrupted upon infection while the other phosphorus syntheses decrease linearly to zero in 10 minutes, the total phosphorus uptake by the coli + T2 culture at the end of 30 minutes would be that found experimentally. The general shape of curve B can be reproduced by assuming a continuation of the DNA synthesis together with an exponential fall during 30 minutes of the other phosphorus uptake to 1 per cent of its value in actively growing E. coli.²

A final set of experiments used ultraviolet-inactivated T2 bacteriophage. The amount of ultraviolet used for this inactivation reduced the number of plaque-forming units about 10^8 times. A typical experiment using UV coli infected with UV T2 is shown in figure 8. In this experiment, the bacterial count per ml before irradiation was 1.2 × 10^8. The number of bacteriophage particles in the final UV coli + UV T2 culture was 6.0 × 10^8 per ml as determined before irradiation. The experiments were made promptly after irradiation of both bacteria and bacteriophage. Curves A and D show as usual the number of plaque-forming units of T2 per ml and of surviving bacteria per ml in the irradiated T2-free control. Curve A represents true multiplication rather than a recovery of the UV T2 since the T2 titer did not increase in broth without addition of the UV coli. Measurements carried out after incubation for longer than 4 hours showed a final T2 titer of about 3 × 10^4 per ml. It is apparent from curves B and C that the presence of ultraviolet-inactivated bacteriophage reduces the phosphorus uptake of the ultraviolet-irradiated bacteria but does not stop it entirely.

² It is emphasized that this speculation is only intended to show that one need not conclude a faster DNA synthesis than is possible with healthy bacteria.
Similar experiments on actively growing *E. coli* infected with UV T2 show a phosphorus utilization intermediate between the total with UV coli + T2 and UV coli alone. The rate of phosphorus uptake in the coli + UV T2 showed a marked reduction about 15 minutes after infection. In a typical instance, the phosphorus utilized during the first 10 minutes (infection ratio 10 to 1) in a sample centrifuged from 50 ml containing $1.4 \times 10^8$ coli per ml before infection was slightly over 10,000 counts per minute. This compares favorably with figure 7a, curve C, where the corresponding uptake was about 6,000 counts per minute when $0.82 \times 10^8$ coli per ml were infected with T2 (infection ratio ca. 10 to 1). The plateau value of ca. 35,000 counts per minute was sufficiently lower than that of curve B of figure 7a to demonstrate that inactivated bacteriophage does arrest phosphorus synthesis in systems involving initially normal bacteria.

The radioactive phosphorus used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, United States Atomic Energy Commission.

**SUMMARY**

The synthesis in nutrient broth of nucleic acids in *Escherichia coli* infected with the T2r+ bacteriophage has been followed with radioactive phosphorus.
When the *E. coli* were irradiated with ultraviolet light before infection, the phosphorus utilized was considerably greater than in the controls of irradiated *E. coli* without T2. The final phosphorus content of ultracentrifugates of the lysate shows good correlation with the final number of T2 plaque-forming units and leads to a phosphorus content per infectious unit that agrees with previously published chemical analyses. The amount of phosphorus in the crude lysates recoverable in the filtered lysates is such as to show that nearly all the nucleic acid synthesized by the infected bacteria is incorporated in mature bacteriophage.

The amount of DNA synthesized by actively growing *E. coli* during one generation lifetime apparently marks an upper limit to the amount of this acid that can become available for bacteriophage production in ultraviolet-inactivated *E. coli*. The data from detailed study of early stages further indicate that the maximum rate of synthesis of DNA in systems involving the infected *E. coli* is approximately the same as in normal actively growing bacteria cultured under similar conditions.

Infection of *E. coli* with ultraviolet-irradiated T2 reduced the phosphorus uptake to an extent that showed that the synthesis of both ribose and deoxyribose nucleic acids had been suppressed. Infection of irradiated *E. coli* with irradiated T2 gave a phosphorus uptake which was less than that for irradiated *E. coli*.

REFERENCES


