INACTIVATION OF BACTERIA BY DECAY OF INCORPORATED RADIOACTIVE PHOSPHORUS*

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INTRODUCTION

Bacteriophages lose their infectivity upon decay of radiophosphorus $^{32}$P incorporated into their deoxyribonucleic acid (DNA). Each inactivation appears to be the consequence of the decay of a single atom of $^{32}$P, although only a fraction of such radioactive disintegrations is actually lethal. By means of reconstruction experiments it is possible to show that the ionizations produced by the hard $^{32}$P $\beta$-electrons on their way out of the virus particles are not the principal cause of this death, but that a "short range" consequence of the radioactive disintegration, like the transmutation $^{32}$P $\rightarrow$ $^{32}$S or the recoil energy sustained by the decaying phosphorus nucleus, must be responsible for the loss of infectivity (1). In the experiments reported here, similar lethal effects on the viability of bacterial cells containing $^{32}$P incorporated into their DNA have been observed. These findings reinforce the conclusions previously reached concerning the mechanism by which DNA macromolecules harboring decaying $^{32}$P atoms are destroyed (2). The localization of these lethal effects within the DNA of the bacterial cell, furthermore, makes possible certain experiments relevant to the function of the bacterial nucleus.

Materials and Methods

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Inhibition of Bacterial Growth at High Specific Activities of P32:

Cultures of Escherichia coli will not grow when inoculated into media containing radiophosphorus P32 at specific activities superior to 500 mc./mg. That this inhibition of bacterial multiplication is produced principally by the decay of P32 atoms which have been assimilated into the cells and not by the radiation emitted by extracellular P32 atoms present in the growth medium has been demonstrated in the following way. A culture of E. coli was inoculated into 2 volumes of H medium, each containing 1.5 mc. of P32 per ml. but differing in total phosphorus content so that their specific radioactivities were 150 and 750 mc./mg. respectively. Periodic microscopic observation of the two cultures during incubation at 37°C showed that bacterial division continued in the low specific activity medium, although some filaments were eventually produced. In the high specific activity medium, on the other hand, there was essentially no increase in the number of cells; many of the bacteria became filamentous and underwent lysis within 3 hours. The rate of P32 assimilation is proportional to the specific activity of the growth medium and hence the bacteria in the high specific activity medium incorporated initially many more atoms of P32 into their phosphorylated constituents per unit time than those in the low specific activity medium. Inasmuch as the growth media of both cultures contained exactly the same total radioactivity, it would follow that the selective inhibition of only the high specific activity culture was due to its higher content of assimilated P32 and concomitant greater frequency of intracellular disintegrations. A calculation shows that in each E. coli cell whose phosphorylated constituents contain P32 at the level of 500 mc./mg. there will occur approximately ten radioactive disintegrations every minute. Hence it seems reasonable to suppose that the failure of cultures to multiply in the presence of very high specific activities of P32 is due to the fact that under these conditions many cells sustain a “lethal” disintegration before they have had time to go through one division cycle. The following experiments, in which the lethal effects of the decay of incorporated P32 on E. coli bacteria have been examined in more detail, substantiate this supposition.

Inactivation of Bacteria by Decay of Incorporated P32:

The bactericidal effect of the decay of incorporated P32 has been studied by storing radioactive bacteria at −196°C. in a frozen, i.e. metabolically inert state. The ability of individual cells to give rise to a colony after various amounts of radioactive decay had occurred was then tested by thawing and plating aliquots of the frozen culture from time to time. All those cells were considered to have been “inactivated” which failed to give rise to a visible colony upon being plated.
H medium, containing $^{32}P$ of specific activity 16 mc./mg., was inoculated with a culture of strain B/r already in its exponential phase of growth in non-radioactive H medium and incubated at 37°C. until the bacterial concentration had increased from $2 \times 10^9$ to $10^9$ cells/ml. The radioactive culture was then diluted into cold GCA medium to reduce the concentration of radioactivity to 0.01 mc./ml. (storage tube). 0.1 ml. aliquots of the storage tube were then frozen and stored in liquid nitrogen ($-196^\circ$C.). From day to day, one of the frozen aliquots was thawed and plated for colony counts. A non-radioactive control culture of B/r was diluted into GCA medium (to which 0.01 mc./ml. $^{32}P$ had been added) and similarly frozen, stored, and assayed. At least 90 per cent of the bacterial cells were recovered as colony formers when a culture was thawed and plated immediately after being frozen.

The results of this experiment are presented in Fig. 1, on which the logarithm of the fraction of bacteria surviving as colony formers has been plotted against
the fraction of \( P^{32} \) atoms decayed at the time of assay. It is apparent that the number of non-radioactive cells capable of forming colonies remained constant, \textit{i.e.} that bacterial survival appeared to be affected neither by prolonged storage at \(-196^\circ\text{C.}\) nor by any irradiation produced by the low level of unincorporated \( P^{32} \) present in the storage tube. Among the radioactive bacteria, on the other hand, a progressively smaller fraction is seen to survive, so that less than 1 in \(10^4\) is capable of generating a colony when 0.8 of the incorporated \( P^{32} \) atoms has decayed. The surviving fraction, \( s_n \), of the radioactive bacteria seems to disappear according to a "multiple hit" curve of the type

\[
s_n = 1 - (1 - s)^n,
\]

as if each cell contained \( n \) "sensitive units" whose surviving fraction \( s \) decreased with the fraction of \( P^{32} \) atoms decayed, \( f \), according

\[
\log s = -Kf
\]

in which \( K \) is a rate constant, and as if the colony-forming ability of the cell was preserved as long as one of these units remained intact. The final slope \( K \) of the survival curve then represents the rate of inactivation of the last sensitive unit per cell and the extrapolation of the final asymptote to zero decay the number, \( n \), of sensitive units per cell. The final asymptote of the survival curve of the radioactive bacteria presented in Fig. 1 extrapolates to the value 3, indicating approximately 3 sensitive units per \( B/r \) cell. (The actual shape of the survival curve deviates slightly from that described by Equation 1; this is probably due to the fact that the bacterial population is not homogeneous with respect to number of sensitive units, some cells containing more and some less than 3 units each.)

\textit{Inactivation of Differentially Labeled Bacteria.—}

Approximately 12 per cent of the phosphorus of \textit{E. coli} cells resides in DNA, the rest forming part of ribonucleic acid (RNA), phospholipides, phosphoproteins, and low molecular weight ("acid-soluble") compounds. If the mechanism of inactivation of bacteria by \( P^{32} \) decay is similar to that by which radioactive bacteriophages lose their infectivity, it is possible that only the decay of radioactive phosphorus atoms incorporated into the bacterial DNA is responsible for loss of colony-forming ability and not the decay of the majority of the intracellular \( P^{32} \) atoms residing in the other phosphorylated cell constituents. The following experiments with \textit{E. coli}, strain 15\textsubscript{T}, a thymine-requiring mutant, show this to be the case. If thymine is removed from the growth medium of 15\textsubscript{T}, the number of viable bacteria in the culture remains constant for a period roughly equivalent to one-half the normal generation time and then decreases rapidly ("thymineless death"). During such thymine starvation, the bacteria increase in size, double their content of RNA but in-
crease in their DNA by no more than 5 to 20 per cent (4, 7). The preferential reduction of DNA synthesis in the absence of exogenous thymine thus provides a means by which relatively more P32 can be introduced into other cellular constituents than into DNA. The rate of inactivation of cultures which contain similar total amounts of P32 per cell but different specific activities in their DNA can then be compared.

A culture of 15T- growing in H medium supplemented with thymine was washed free of thymine and incubated for 10 minutes at 37° in thymine-free H medium. Two aliquots of radioactive H medium of specific P32 activity 280 mc./mg., one containing no thymine and one containing 5 mg./ml. thymine, were then inoculated with these bacteria to a density of 5 X 10^6 cells/ml. After incubation at 37° for 2 and 30 minutes, samples of both cultures were diluted into cold GCA medium (storage tubes). The storage tubes were then frozen, stored, and thawed and plated from day to day for colony formers, as above. The amount of radiophosphorus assimilated by the two cultures during their incubation in the radioactive H media was measured as trichloroacetic acid (TCA)-insoluble P32. For this purpose, an aliquot of each storage tube was added to an equal volume of cold 0.6 作文 TCA, containing 2 X 10^8 non-radioactive carrier bacteria per ml. Unincorporated and TCA-soluble P32 were then removed by centrifugation and washing in the cold. The TCA-insoluble material was dissolved in 1 N NaOH and its content of P32 counted.

The results of this experiment are presented in Table I where it is seen that the titer of viable bacteria in the radioactive culture containing thymine increased by 20 per cent between the 2nd and 30th minute of incubation whereas the titer of viable bacteria in the thymine-free culture decreased by 40 per cent during the same time. This difference in final titer reflects, on the one hand, the growth of the culture in the presence of thymine and, on the other hand, the onset of "thymineless death" in the absence of thymine. The amount of TCA-insoluble P32 which the one culture had assimilated in the absence of thymine was only 20 per cent less than that which the other had assimilated in the presence of thymine. The survival after P32 decay of the two cultures incubated

<table>
<thead>
<tr>
<th>Growth medium (As = 280 mc./mg.)</th>
<th>Time of incubation</th>
<th>Relative titer of viable bacteria</th>
<th>TCA-insoluble P32 (cts./min./ml. of culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With thymine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min.</td>
<td>1.0</td>
<td></td>
<td>6.6 X 10^6</td>
</tr>
<tr>
<td>30 min.</td>
<td>1.2</td>
<td></td>
<td>2.2 X 10^6</td>
</tr>
<tr>
<td>Without thymine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min.</td>
<td>1.0</td>
<td></td>
<td>6.0 X 10^6</td>
</tr>
<tr>
<td>30 min.</td>
<td>0.6</td>
<td></td>
<td>1.8 X 10^6</td>
</tr>
</tbody>
</table>
for 30 minutes is presented in Fig. 2a, on which the logarithm of the fraction of individuals capable of generating a colony after thawing is plotted against the fraction of $^{32}$P atoms decayed. It is seen, first of all, that although the time of incubation of both cultures was only 30 minutes, more than 99 per cent of the cells of both cultures are subject to inactivation by $^{32}$P decay. The culture which had assimilated its $^{32}$P in the presence of thymine, however, is inactivated much more rapidly than that which had assimilated its $^{32}$P in the absence of exogenous thymine. In fact, the maximum slope of the inactivation curve of

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**Fig. 2a.** $^{32}$P inactivation of E. coli 15T- at $-196^\circ$C. after incubation for 30 minutes in radioactive media ($A_0 = 280$ mc./mg.) with and without thymine. b. Effect of thymine starvation on $^{32}$P inactivation of 15T- ($A_0 = 16$ mc./mg.) at $-196^\circ$C. Triangles, radioactive bacteria subjected to 40 minutes' thymine starvation in radioactive medium ($A_0 = 16$ mc./mg.) prior to storage. Circles, radioactive bacteria incubated for 40 minutes in radioactive medium ($A_0 = 16$ mc./mg.) with thymine before storage.
It is conceivable that the great difference in rate of inactivation of the two cultures presented in Fig. 2 a could be due to some physiological effect produced by the 30 minute thymine starvation to which one of the cultures was subjected, an effect which might be thought to have reduced the inherent sensitivity of the 15T-bacteria to inactivation by decay of assimilated P32. To test for this possibility, a culture of 15T- was grown in thymine-supplemented, radioactive H medium containing P32 at 16 mc./mg. After a 50-fold multiplication over the inoculum had occurred, the bacteria were washed free of thymine and 2 aliquots incubated for 40 minutes at 37° in H medium of the original specific activity, one in the presence and the other in the absence of thymine. Both aliquots were then diluted in cold GCA medium (storage tube). The storage tubes were then frozen, stored, and thawed and plated from day to day for colony formers as above.

The results of this experiment are presented in Fig. 2 b, in which it may be seen that the initial rate of inactivation of the thymine-starved culture, was, in fact, greater, not less, than that of the culture which had never been deprived of thymine. The final rates of inactivation of both cultures however, are seen to be identical. It would appear, therefore, that the fourfold difference in the rate of inactivation of the two cultures presented in Fig. 2 a is not due to a reduction of the P32-sensitivity produced by thymine starvation. The reduced rate of inactivation of the cells which had assimilated their P32 in the absence of thymine must, rather, be due to the fact that very little of this P32 was introduced into the bacterial DNA. Consequently, it may be inferred that it is the decay of the DNA-radiophosphorus atoms which is mainly responsible for the loss of the colony-forming ability of the bacterial cells. It may be concluded, furthermore, that, as in the case of the P32 inactivation of bacterial viruses, a "short range" effect of the radioactive disintegration is responsible for death and not the β-radiation concomitantly emitted. For the range of the β-electrons emitted by P32 is so much longer than the dimensions of the E. coli cell, that were β-ionizations mainly responsible for bacterial death, no difference in lethal efficiency could exist between β-electrons originating in the DNA and those originating in other cellular constituents. The fact that the thymineless culture of Fig. 2 a is inactivated at all is compatible with the idea that this inactivation is due to those P32 atoms which found incorporation into DNA in the course of the small residual DNA synthesis which may take place even in the absence of thymine (4).
It has been observed in cytological studies that, depending on the conditions of growth, the “most frequent” number of nuclei possessed by cells of strain B/r is between 2 and 4 (3). It would seem, therefore, that the 3 sensitive units per cell inactivated separately by P³² decay in the radioactive B/r culture of Fig. 1 are the bacterial nuclei. Each cell appears to be capable of giving rise to a colony as long as P³² decay has left at least one of its nuclei intact. The final slope K of the inactivation curve of Fig. 1 then represents the rate of inactivation of individual nuclei by decay of P³² atoms they contain. It can be shown (1) that the rate constant K has the value

\[ K = 1.48 \times 10^{-6} \alpha_{32} A_0 N \]  

in which \( A_0 \) is the initial specific activity of the DNA–P³², \( N \) the number of DNA–phosphorus atoms per nucleus, and \( \alpha \) the “efficiency of killing,” i.e.,

<table>
<thead>
<tr>
<th>Material</th>
<th>( A_s )</th>
<th>Final slope of death curve, ( K )</th>
<th>DNA-P per cell</th>
<th>Nuclei per cell</th>
<th>DNA-P per nucleus</th>
<th>( N ) atoms of DNA-P per nucleus or phage</th>
<th>( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/r</td>
<td>16</td>
<td>-8.3</td>
<td>180 ( \times 10^{-14} )</td>
<td>3</td>
<td>60 ( \times 10^{-14} )</td>
<td>120 ( \times 10^3 )</td>
<td>0.02</td>
</tr>
<tr>
<td>T3</td>
<td>160</td>
<td>-1.6</td>
<td>2 ( \times 10^4 )</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
</tbody>
</table>

The results of these determinations and calculations are presented in Table II, in which not only the final slope \( K \) of the inactivation curve of the radioactive B/r of Fig. 1 but also the final slopes of two other inactivation experiments with more highly radioactive B/r cultures are listed. It is seen that, as demanded by equation (3), the rate of inactivation of individual nuclei \( K \) in the three experiments is roughly proportional to the specific activity \( A_s \). The final column lists the efficiency of killing \( \alpha \), estimated on the basis of the three inactivation experiments, which is seen to be 0.02 “hits” per P³² disintegration per nucleus. In other words, one out of every fifty P³² disintegrations taking place at \(-196^\circ\) appears to inactivate the B/r nucleus in which it has occurred. For
comparison, the data for an efficiency of killing calculation for the coliphage strain T3 are also presented in Table II, for which \( \alpha \) is seen to be 0.04 "hits" per \( P^{32} \) disintegration per phage. The efficiencies of killing in these two rather different biological objects are, therefore, seen to be of the same order of magnitude.

On the basis of equation (3) and the values of the rate constants \( K \) given in Table II, one may now estimate that in B/r cells containing more than 500 mc./mg. \( P^{32} \) there should be on the average more than one lethal disintegration per nucleus every hour, the division time of strain B/r in radioactive H medium at 37\(^\circ\). Since the efficiency of killing could be expected to be even greater at 37\(^\circ\) than at \(-196^\circ\) (2), it is apparent that \( E. coli \) cultures are indeed no longer able to multiply at high specific activities because most of the cells suffer inactivation before having had time enough to reproduce themselves.

**Multiplication and Thymineless Death of Bacteria after \( P^{32} \) Decay.**

It has been seen that a number of nuclei must apparently be inactivated by \( P^{32} \) decay in each bacterium before the ability of the cell to give rise to a clone of descendants, i.e. to a colony, is finally destroyed. Two further properties of \( 15\tau_\gamma \) populations have now been examined at various stages of \( P^{32} \) decay: (a) the multiplication of the survivors in a medium which contains thymine and (b) the inactivation of the survivors by thymine starvation in a thymine-free medium (thymineless death).

Two cultures of \( 15\tau_\gamma \) were grown in thymine-supplemented H medium, one in the presence of \( P^{32} \) at 16 mc./mg. and the other in the absence of any \( P^{32} \). After growth, the cultures were diluted into cold GCA medium and the storage tubes frozen, stored, and thawed and plated from day to day for colony formers, as above. At various stages of \( P^{32} \) decay, furthermore, thawed aliquots of both radioactive and non-radioactive cultures were diluted either into H medium containing 2 \( \gamma \)/ml. thymine or into thymine-free H medium, then incubated at 37\(^\circ\) and plated for colony formers after various times of incubation.

The results of this experiment are presented in Fig. 3 a and 3 b, on which the fraction of the original number of cells in each culture capable of giving rise to a colony is plotted against the time of incubation at 37\(^\circ\). The intercept of each curve with the ordinate of zero incubation time in the two figures represents the fraction of the original population "surviving" the amount of \( P^{32} \) decay which had taken place by the day on which the sample was thawed. It is seen in Fig. 3 a that the non-radioactive culture when thawed either soon after being frozen or after being stored for 3 or 6 days resumed its multiplication without any appreciable delay when transferred to 37\(^\circ\) in the presence of thymine. In other words, immediately after thawing, the bacteria appear to resume their activities where they had left them off at the moment of freezing. The
radioactive culture, on the other hand, already exhibited a 40 minute lag before multiplication resumed when it was thawed after only a few hours' storage at \(-196^\circ\), at which time there had been little or no inactivation of its colony-forming ability. When the radioactive culture was thawed after 3 days' storage,

![Graph](https://i.imgur.com/3J5Q5Q5.png)

**Fig. 3 a.** Multiplication in thymine-supplemented medium and b thymineless death in thymine-free medium of radioactive and non-radioactive cultures of *E. coli* 15T- after storage for various times at \(-196^\circ\). Time of storage: circles < 0.5 day; upward triangles, 3 days; downward triangles, 6 days. Filled symbols, radioactive culture \((A_0 = 16 \text{ mc./mg.}); open symbols, non-radioactive culture. Ordinate, colony counts relative to original bacterial population. Abscissa, time of incubation at 37°C. after thawing.

when 30 per cent of the initial population was still capable of colony formation, the division lag of the survivors increased further to 80 minutes. Finally, after 6 days' storage, when only 2 per cent of the radioactive 15T- cells were still capable of giving rise to a colony, 110 minutes at 37°C had to elapse before the thawed culture manifested any signs of multiplication.
The multiplication lag of the bacteria surviving $^{32}P$ decay reflects some “non-lethal” damage wrought by the intracellular radioactive disintegrations. Since in the course of $^{32}P$ decay the number of intact nuclei per surviving cell is thought to be reduced until there remains only one, it would appear reasonable to attribute some of the observed multiplication lag to the time required for the cell to regenerate a sufficient number of functional nuclei with which viable daughter cells can be endowed. It is difficult, however, to explain entirely on this basis the further increase in lag from 80 to 110 minutes while the fraction of $^{32}P$ survivors decreases from 0.3 to 0.02, since this part of the inactivation is already proceeding according to the “exponential” part of the multiple hit survival curve, when the surviving bacterial population should finally be “mononucleate.” Possibly the necessity for repair of some additional non-lethal, cytoplasmic damage is responsible for this further increase in lag. It should be noted that the multiplication of the survivors of $^{32}P$ decay appears to take place in a stepwise manner, not unlike the “synchronous” growth of $^{15}$T- observed among the survivors of short periods of thymine starvation (11). The possibility of achieving synchronous growth by $^{32}P$ decay has, however, not been examined with sufficiently great precision to make this observation statistically significant.

The effect of thymine starvation on radioactive and non-radioactive cultures after various lengths of storage at $-196^\circ$ is presented in Fig. 3 b. It is seen that the three aliquots of non-radioactive $^{15}T$- cells stored in the frozen state for various times all exhibit the typical “thymineless death” upon growth in thymine-free medium, i.e. constant colony count for the first 25 minutes followed by rapid loss of colony formers, leaving only 10 per cent of the initial population viable at the 55th minute. The survivors of the radioactive $^{15}T$- cultures are likewise seen to be subject to thymineless death, but it is to be noted that the period of constant cell count appears to be reduced and the onset of loss of colony formers hastened by prior $^{32}P$ decay. In the culture subjected to only a few hours of $^{32}P$ decay, thymine starvation for 45 minutes left 10 per cent of the initial colony formers surviving. In the cultures stored for 3 and 6 days, in which $^{32}P$ decay had left respectively 30 and 2 per cent survivors, thymine starvation for only 30 minutes following thawing sufficed to reduce the count of viable cells by another factor of ten. The rate, however, at which thymineless death proceeds, once under way, appeared to be unaffected by any previous $^{32}P$ decay. If the result presented in Fig. 2 b is recalled, in which it was observed that prior thymine starvation hastened the initial rate at which $^{15}T$- cells are then inactivated by $^{32}P$ decay, though leaving the ultimate or exponential part of the $^{32}P$ survival curve unaffected, it becomes evident that $^{32}P$ decay and thymine starvation cooperate in bringing about the death of bacterial cells as colony formers. A preliminary treatment by one reduces the amount of subsequent treatment required to produce the maximum inactivation rate by the other. Since the lethal effect of $^{32}P$ decay has been localized
within the DNA, it seems plausible to infer from these observations that the lethal effect of thymine starvation is likewise an inactivation of bacterial nuclei. When, therefore, either P\textsuperscript{32} decay or thymine starvation has already inactivated some of the nuclei of a surviving 15\textsuperscript{r} cell, subsequent starvation of or decay in such a population will eliminate the remaining intact nuclei, and hence the ability of the cell to give rise to a colony, after a shorter induction period. It should be emphasized here that the actual mechanism by which the bacterial nucleus is affected and its physiological and biochemical potentialities after inactivation could be entirely different following the two types of treatment. Required under this view is only that a nucleus “inactivated” in either manner is no longer capable of regenerating an indefinite succession of daughter nuclei for a clone of viable descendants.

**Distribution of the DNA-Phosphorus Atoms of Bacteria among Daughter Cells.**

It has been shown that the DNA of *E. coli* cells retains its phosphorus atoms throughout subsequent bacterial growth and multiplication (12, 13). How are these phosphorus atoms distributed over the nuclei of daughter cells? Do some descendant nuclei contain only atoms assimilated *de novo* and are others endowed exclusively with phosphorus atoms of parental origin, or are the atoms of the parental nucleus dispersed among all the nuclei in its line of descent? The fact that it is the decay of DNA-P\textsuperscript{32} atoms which is mainly responsible for the death of the bacterial cells offers a method of resolving this question. For if in the course of their reproduction the parental nuclei preserve their atomic integrity, *i.e.* if newly assimilated phosphorus atoms are introduced only into the progeny structures, then the stable nuclei of a non-radioactive culture of cells inoculated into a medium containing P\textsuperscript{32} would never acquire any radioactivity and hence remain stable. After growth in the radioactive medium, the culture would still possess its initial number of non-radioactive nuclei and the colony-forming ability of cells harboring these nuclei could not be eliminated by P\textsuperscript{32} decay. Conversely, when a radioactive culture, homogeneously subject to loss of colony-forming ability by radioactive decay, is inoculated into a medium containing no P\textsuperscript{32} and if the nuclei subsequently synthesized contain only *de novo* material, then very quickly a class of cells will arise which contain entirely non-radioactive, *i.e.* stable nuclei and which should be refractory to inactivation by decay. If, on the other hand, the atomic identity of the nucleus is destroyed in the course of bacterial proliferation and the parental atoms dispersed among the daughter structures, then in the two experiments just considered stable nuclei of non-radioactive cells will quickly become radioactive upon growth in the presence of P\textsuperscript{32} and radioactive nuclei will still give rise to radioactive, albeit *less* radioactive, nuclei upon growth in the absence of P\textsuperscript{32}.

H medium containing P\textsuperscript{32} at 200 mc./ml. was inoculated with growing, non-radioactive B/r bacteria to a final density of 10\textsuperscript{8} cells/ml. This radioactive culture was
The results of the first part of this experiment are presented in Fig. 4, in which the survival of the non-radioactive B/r culture, which has undergone various numbers of divisions in radioactive growth medium, is shown as a
function of the fraction of P\textsuperscript{32} atoms decayed. The parental culture, having not yet made any divisions, is seen to be stable. After 1.6 divisions in the presence of P\textsuperscript{32}, however, at least 99.9 per cent of the bacterial cells had become sensitive to inactivation by P\textsuperscript{32} decay. After 3.6 and 4.4 divisions, the populations are seen to have experienced a further increase in sensitivity to inactivation. The slope of the exponential part of the inactivation curve after 1.6 divisions is 70 per cent of the final slope after 4.4 divisions, in harmony with the estimate that after 1.6 divisions approximately 66 per cent of the bacterial DNA has been synthesized de novo. It, therefore, appears that the atomic identity of the bacterial nucleus is not preserved in the course of its reduplication but that parental and newly assimilated DNA–phosphorus atoms become intermingled within

\[ \sigma(D) = 1 - [1 - \exp(-Kf/2^D)]^n. \]
daughter nuclei. The results of the second part of this experiment are presented in Fig. 5, on which the survival of the radioactive B/r culture, which have undergone various numbers of divisions in non-radioactive medium, is plotted against the fraction of $^{32}$P atoms decayed. The parental culture, having not yet undergone any divisions in the absence of $^{32}$P and being identical to the 4.4 divisions culture of Fig. 4, is seen to be subject to rapid death. The cultures having undergone 1.4, 2.8, and 3.9 divisions respectively are seen to have become progressively less sensitive to inactivation, but in none of these populations had there appeared a detectable fraction of completely stable cells. The survival of the culture after various numbers of divisions, expected on the basis of an equipartition of the parental atoms at each nuclear duplication has also been indicated in Fig. 5. These theoretical survival curves were calculated by a "dose-reduction" principle, i.e., by multiplying the abscissae of the survival curve of the original radioactive parental population by the factor by which the number of cells in the culture had increased over the inoculum. A comparison of the observed with the theoretical survival curves shows that after 1.4 divisions the population is inactivated as though the $^{32}$P of the parental DNA is equidistributed among daughter nuclei. After 2.8 and 3.9 divisions, however, the survival curves depart more and more from those predicted by equidistribution. It is possible that this departure is the reflection of the appearance of an asymmetry in the partition of the DNA phosphorus atoms during the second but not during the first nuclear reduplication following their assimilation. An equally likely explanation would be, however, that the slowly dying tail of the later survival curves represents the descendants of a more rapidly growing minority of the bacterial population, which had undergone more divisions than the average and had, therefore, reduced its specific radioactivity content more than estimated by the dose-reduction calculation. In any case, the second part of this experiment confirms the conclusion already reached from the results of the first, i.e., that the phosphorus atoms of the parental nucleus become dispersed among its descendants. This conclusion is in harmony with the observation that the phosphorus atoms of the DNA of T2 and T4 bacteriophage particles are likewise dispersed, though not equidistributed, over their progeny (14, 15). It is, unfortunately, not possible to infer from the present experiments the mechanism by which this dispersion occurs, i.e. whether it is due to a partition of the parental atoms in the course of the elementary replication act of the DNA itself, such as demanded by some proposals concerning this process (16, 17) or whether it is due to the randomizing effect of some postreplication event, such as "crossing-over" or assortment of "chromosomes."

**DISCUSSION**

It has been observed that the efficiency of killing $\alpha$ with which decay of incorporated $^{32}$P atoms destroys the infectivity of bacteriophage particles is
very nearly the same for a number of bacteriophage strains which differ greatly in their size, chemical constitution, morphology, genetic structure, and manner of interaction with bacterial host cells. This efficiency on the other hand varies with the temperature at which decay proceeds, rising from 0.04 at -196°C to 0.3 at +65°C (2, 18, 19). On the basis of these findings it was concluded that the efficiency $\alpha$ must reflect some structural aspect of the DNA macromolecule rather than of the virus particles. A mechanism was suggested in terms of the two-stranded helical Watson-Crick structure of DNA (20) to account for the manner in which decay of incorporated $^{32}$P affects the integrity of the molecule harboring the disintegrating atoms. It was proposed that the high proportion of non-lethal decays reflected the possibility that the physiological function of the DNA molecule could be preserved even after radioactive decay had interrupted one of the single polynucleotide strands. The lethal decays were thought to be only those which had resulted in a complete cut of a DNA double helix (2).

The discovery that one "lethal" $^{32}$P disintegration suppresses the survival of only a part of the genome of an inactivated bacteriophage particle has lent further support to the notion that the lethal effect is localized within a single macromolecule (21, 22).

The finding reported here that bacterial nuclei appear to be inactivated by decay of incorporated $^{32}$P with an efficiency of killing of the same order of magnitude as bacteriophages strengthens these views concerning the mechanism of damage. For the great difference in size, structure, and function which must exist between $\textit{E. coli}$ nuclei and bacteriophages makes it unlikely that any factor other than the similarity of the basic structure of the DNA molecules of both could be responsible for so comparable a fraction of the $^{32}$P disintegrations being lethal. (The difference between the efficiency $\alpha$ actually found in B/r (0.02) and that observed for T3 (0.04) at the same temperature, cannot be considered very significant, in view of the possible multiplicative errors inherent in the determinations of total amount of DNA phosphorus per cell, number of "sensitive units" per cell, and specific activity of growth media. This discrepancy might, nevertheless, be the reflection of some genuine structural difference.) The present observations, finally, confirm the earlier conclusions that $\beta$-electron ionizations are not mainly responsible for the lethal effects of incorporated $^{32}$P.

The two applications of $^{32}$P decay in bacteria presented here, i.e. the mechanism of "thymineless death" and the partition of parental DNA phosphorus atoms among daughter cells, would appear to be only examples of a larger class of experiments susceptible of exploitation by this technique. Many other problems in which it is desired to investigate the role of the bacterial nucleus in some aspect of metabolism or physiology could possibly be resolved by examination of radioactive bacteria before and after $^{32}$P decay. Such experiments concerned with the capacity of bacteria to reproduce infecting bacteriophages
or to synthesize induced enzymes have already been carried out and will be reported elsewhere.

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SUMMARY

Cultures of Escherichia coli will not grow in media containing very high specific activities of radiophosphorus $^{32}P$, the inhibition of growth being due to the decay of assimilated $^{32}P$ atoms. Experiments with a differentially labeled thymineless strain of E. coli show that the $^{32}P$ disintegrations which occur in the bacterial deoxyribonucleic acid, i.e. in the nucleus, are mainly responsible for the inactivation of the cell. The kinetics with which radioactive bacterial populations are inactivated indicate that the function of several nuclei per bacterial cell must be eliminated by $^{32}P$ decay before the ability to generate a colony is lost. The efficiency with which each $^{32}P$ disintegration inactivates the nucleus in which it has occurred is calculated to be 0.02 (at $-196^\circ$), i.e., similar in magnitude to the killing efficiency of $^{32}P$ decay in bacteriophages.

$^{32}P$ decay and thymine starvation cooperate in bringing about the death of individuals of the thymineless strain, from which observation it is inferred that "thymineless death" is likewise a nuclear inactivation.

The descendants of a non-radioactive bacterial culture grown for several generations in the presence of $^{32}P$ and the descendants of a radioactive culture grown in the absence of $^{32}P$ are inactivated by $^{32}P$ decay in a manner which indicates that the phosphorus atoms of bacterial nuclei are dispersed among the progeny nuclei in their line of descent.

REFERENCES