IN VIVO REPAIR OF UV IRRADIATION DAMAGE OF SINGLE STRANDED DNA PHAGE \( \Phi X174 \)

3.01 Introduction

Any ionising radiation or ultraviolet light can inactivate bacteria and bacteriophage (Setlow, 1966). Here inactivation means inability to form colonies or plaques under suitable growth condition. Hollander and Claus (1937) first showed positive indication of recovery from radiation injury by observing that UV irradiated fungal spores gave higher survival, if these were held in liquid before plating, instead of plating them immediately after irradiation. Since then several types of radiation recovery was observed, but wide general interest in this field arose with the observation of photoreactivation (PR) in the condi of Streptomyces griseus, Escherichia coli, Penicillium notatum and yeast cells (Kelner, 1949 a & b) and its almost simultaneous finding by Dulbeco (1949) in bacteriophage. PR is the recovery of a biological system from the effects of ultraviolet light irradiation as a result of post irradiation treatment with light of longer wavelength usually in the visible range. This phenomenon has been observed in wide range of microorganisms such as viruses bacteria, algae, fungi, protozoa, etc. (for review see Rupert & Harm, 1966).

Garen and Zinder (1955) showed that a higher survival curve of UV irradiated Salmonella phage P22 could be obtained.
if the host cells preirradiated with UV or X-rays. This reactivation mechanism of irradiated phage in irradiated bacteria usually referred to as UV-reactivation (UVR), was also found in \( \lambda \) (Weigle, 1953), T3 (Weigle & Dulbecco, 1953), T1 (Tessman, 1956) and HP1 (Harm and Rupert, 1963).

Another type of reactivation called host cell reactivation (HCR) was found in \( \lambda \) and other temperate phages as well as in T1, T3, T7 (for review see Rupert & Harm, 1966). HCR process, as its name implies, occurs in unirradiated bacteria instead of irradiated bacteria as in the case of UVR. HCR or UVR was not observed with T3 and T-even phage. But T2 was more UV sensitive than T4. According to Harm (1959, 1961) the increased UV sensitivity of T2 was due to absence of U-gene, which was present in T4 phage and was able to restore UV lesions in the phage DNA.

A number of considerations argued that the most harmful events due to UV irradiation were photochemical reactions in nucleic acids (for review see Haynes, 1964; Setlow, 1966; Setlow, 1967; Hutchison, 1966). It had long been known that the action spectra for inactivation of bacteria and viruses resemble the absorption spectra of nucleic acids (Zelle & Hollaender, 1955; Setlow, 1957). Nucleic acids were also highly UV sensitive as shown by studies of infectious viral DNA (Sinsheimer, Starman, Nagler & Guthrie, 1962; Harm & Rupert, 1963); infectious viral RNA
UV irradiation of DNA would cause chain breaks, denaturation, crosslinks, formation of hydrates of cytosine and uracil and pyrimidine dimers (for review see Setlow, 1967). But dose required to produce the above changes except pyrimidine dimers was very much higher than usually required for producing biological damage. Within ultraviolet range 200-300 μm, the main absorption took place in the pyrimidine bases of the nucleic acids and the main photoproducts were pyrimidine dimers and hydrates in which water was added across the 5-6 double bond. The hydrate of cytosine was very unstable and rapidly converted to cytosine. Pyrimidine dimers were formed between two adjacent bases by fusing two molecules through a cyclobutane ring at the 5-6 double bonds.

The dimer of thymine was first isolated and identified by Beukers and Berends (1960). Wacker, Dellweg and Weinblum (1960) isolated thymine dimers from DNA irradiated in vivo and showed a correlation between thymine dimerization and bacterial survival. Setlow and Setlow (1962) also obtained evidence that thymine dimers were a cause of UV damage in cells. The observation of Stahl, Crasemann, Okun, Fox and Laird (1961) and of Opara-Kubinska, Lorkiewicz and Szybalski (1961) that replacement of thymine by 5-bromouracil markedly increased the radiation sensitivity of the organism
THYMINE DIMER
\[ T + T \xrightarrow{h\nu} T \]

URACIL DIMER
\[ U + U \xrightarrow{h\nu} U \]

URACIL

HYDRATE OF URACIL
\[ U \xrightarrow{h\nu + H_2O, heat} U^* \]

CYTOSINE

HYDRATE OF CYTOSINE
\[ C \xrightarrow{h\nu + H_2O} C^* \]
and reduced the reactivating capacity also suggested that thymine dimers were the major lethal photoproducts in DNA.

All the recovery processes of an irradiated organism were supposed to be enzymatic in nature (for review see Howard-Flanders, 1968; R.B. Setlow, 1968) and their action might overlap each other. About 85% of the lethal UV lesion occurring in T1 were host cell reactivable, about 80% were photoreactivable and about 95% were reactivable by combined HCR and PR (Ellison, Feiner & Hill, 1960). Photoreactivation of E. coli cells was supposed to be due to the presence of an photoreactivating enzyme in cell extracts (Rupert, 1965). Photoreactivation of biological properties had been correlated with the destruction of dimers in vivo and it was demonstrated in E. coli (Setlow, Swenson & Carrier, 1963), and in Bacillus megaterium (Donnellan & Stafford, 1968). Crude extract of yeast cells plus visible light was able to eliminate thymine dimers from irradiated DNA in vitro (Setlow, 1969; Rupert, 1962; Wacker, 1961).

Among the reactivation mechanism host cell reactivation has been studied most extensively. The idea of genetic control of HCR mechanism came about with the discovery by Hill (1958) of an exquisitely sensitive mutant of E. coli, designated E. coli Bs-1. Ellison, Feiner and Hill (1960) demonstrated that the survival of UV irradiated bacteriophage T1 and T3 was greatly reduced, if plated on E. coli Bs-1...
instead of *E. coli* B. This mutant (hcr") was said to have lost the capacity for HCR. Howard-Flanders, Simson and Theriot (1964), Van de Putte, Van Sluis, Van Dillewijn and Rorsch (1965), and Howard-Flanders, Boyce and Theriot (1962) isolated UV-sensitive mutants of *E. coli* K12 that were also hcr" and found the mutations to map in three widely spaced genetic loci, *uvrA*, *uvrB* and *uvrC*. A mutation at any one of the three *uvr* loci can cause the loss of capacity to reactivate DNA containing UV photoproducts.

Setlow and Carrier (1964), Howard-Flanders and Boyce (1964) proposed a theory for the mechanism of enzymatic repair of DNA containing UV photoproducts in UV irradiated bacteria on the basis of the following experiments. Boyce and Howard-Flanders (1964) incubated two strains of *E. coli* K12, one sensitive and the other resistant, after UV irradiation. It was found that, although equal amounts of thymine dimers were formed in both cases, in UV resistant cells the dimers appeared in the acid soluble fraction i.e., disappeared from the polynucleotide chain and DNA synthesis resumed after an interval, but this did not occur in sensitive non-reactivating mutant; in the latter case, thymine dimers were found in acid insoluble fraction, i.e. with the polynucleotide chain and block in DNA synthesis continued. Setlow and Carrier (1964) showed a similar excision of thymine dimers in strain B/r, but not in B_{s-1}. Since the block of DNA synthesis caused by thymine dimer was removed
in resistant cells and since the cells could form colonies, it was reasonable to assume that once the block of DNA synthesis was removed the molecular events associated with repair of damage to DNA had been completed. Pettijohn and Hanawalt (1964) showed direct physical evidence for repair replication step. UV irradiated cultures of thymine-requiring bacteria were grown in presence of the thymine analogue, 5-bromouracil. Density distribution analysis in the CsCl density gradient of the isolated DNA indicated that the density label was incorporated into very short segments along single DNA strands.

From the above, steps involved in host cell reactivation were proposed to be as follows: (1) excision and removal of oligonucleotides containing UV photoproducts, mainly thymine dimers. An enzyme capable of excising thymine dimers from UV-irradiated DNA has recently been isolated by Kaplan, Kushner and Grossman (1969); (2) resynthesis of missing nucleotide sequence. The enzyme, DNA polymerase (Richardson, Inman & Kornberg, 1964) was most probably required for the step; (3) rejoining of the newly synthesized sequence with the broken phosphodiester backbone which can join two DNA fragments through phosphodiester linkage. An enzyme, called polynucleotide ligase, capable of carrying out this step, has also been discovered (Gellert, 1967; Olivora and Lehman, 1967).
Bacteriophage \( \Phi X174 \), S13 or \( \Phi R \) which contain single stranded DNA as their genetic material, have so far been found not to undergo any host cell reactivation i.e., their UV irradiation damage is not repaired even by her\(^+\) cells which can repair such damage of double stranded DNA phage like T1, T3, or \( \lambda \) (Rorsch, 1963; Ono & Shimazu, 1966). Jansz, Pouwels and Van Rotterdam (1963), and Yarus and Sinsheimer (1964) have also reported that host cell reactivation is absent for UV irradiated free ssDNA of bacteriophage \( \Phi X174 \), although the UV damage of its corresponding double stranded 'replicative form' RF is host cell reactivable. We describe here an experimental condition which allowed the damage sustained by UV irradiated ssDNA phage, \( \Phi X174 \), to be repaired \textit{in vivo} by appropriate host cells and suggest a mechanism by which such repair can take place.
3.02 Experimental Methods

3.02 a Ultraviolet Irradiation

The ultraviolet source was a 15 watt GE germicidal lamp (G 15 T8). Samples were irradiated in 2.5 ml 0.1 M tris buffer, pH 7.4, in 5 cm petri-dishes at ice temperature at a distance of 30 cm from the source. All experiments were performed in dim yellow light or in complete darkness to prevent photoreactivation.

3.02 b Extraction of DNA

$^{32}$P labeled $\Phi X174$ was prepared as described in Sec.(1.02 a), specific activity of labeling being 100 mc/mg P. Free ssDNA was extracted from $^{32}$P labeled $\Phi X174$ by hot phenol method of (Guthrie & Sinsheimer, 1963). Freshly distilled phenol was saturated with 0.1 M sodium tetraborate was brought to 70°C, phage suspension containing 1 mg/ml bovine serum albumin at 70°C was added to phenol in equal proportion, shaken mildly in wrist action shaker, reheated to 70°C alternately for 3 min and then centrifuged at about 5,000 rpm for 5 min. Aqueous layer was separated and extracted twice more with phenol at room temperature. Finally phenol was removed by repeated ether extraction in cold and ether was removed by bubbling air through the sample. $\Phi X$ DNA was precipitated by the addition of 0.1 vol of acetate-EDTA and 2 vol of isopropanol. The precipitate was suspended in 1/10 dil SSC.
To obtain RF DNA, bacteria infected with $^{32}$P-labeled \( \Phi X174 \) were centrifuged and washed in saline-EDTA, and then incubated with 0.5% sodium dodecyl sulphate at 37°C for 10 min. The lysed complexes were digested with 1 mg pronase per ml for 1 hr and shaken with freshly distilled phenol saturated with 0.01 M sodium tetraborate for 40 min. Phenol was removed by repeated ether extraction from the aqueous layer to which was added 0.1 volume acetate-EDTA and 2 volume isopropanol, and kept in cold overnight. The precipitated DNA was finally dispersed in SSC. Recovery of DNA extracted from complexes as measured by radioactivity counts was found to be 70 to 80 per cent in all cases.

3.02 C Sucrose Density Gradient Sedimentation

A 0.2 ml sample of DNA, prepared as above, was loaded on the top of 4.8 ml 5 to 20% sucrose (v/v) gradient in 1/10 dilute SSC and centrifuged at 37,000 rpm for 6 hr at 4°C in SW39 rotor of Spinco model L preparative ultracentrifuge. Velocity sedimentation in neutral low salt sucrose gradient was used to enhance the resolution between the linear and circular forms of ssDNA (Goulian, Kornberg & Sinsheimer, 1967). After the run the tube was pierced at the bottom and three drops apiece were collected in 1.5 ml SSC. Radioactivity counts of the fractions were taken with a G.M. counter. The s-values of radioactivity peaks were assigned with respect to the position of a sample of \( \text{E. coli} \) DNA, having an s-value of 24 as determined in the
Spinco model E analytical ultracentrifuge, and run in exactly identical conditions as above.

3.03 Results

3.03 a Growth of \( \Phi X174 \) in \( E. coli \) \( C_{syn}^- \)

To find the latent period and burst size of \( \Phi X174 \) when \( E. coli \) \( C_{syn}^- \) was used as host bacteria, one step growth experiment described by Adams (1959) was performed. 10 ml tryptone broth was inoculated with overnight grown \( E. coli \) \( C_{syn}^- \) and incubated with aeration until bacterial concentration reached \( 2-3 \times 10^8 \) cells/ml. The bacteria were centrifuged, washed with starvation buffer and starved in the same buffer for one hour. The starved bacteria were infected with \( \Phi X174 \) at m.o.i = 0.1, allowed 15 min for adsorption and centrifuged to discard unabsorbed phage. Phage infected complexes were suspended in tryptone broth and diluted \( 10^2 \) x (DIL tube), \( 10^4 \) x (GT-1 tube), and \( 10^6 \) x (GT-2 tube) in the same broth. The complexes were allowed to grow at \( 37^\circ C \) with aeration and infective centers were assayed from GT-1 and GT-2 tubes at regular interval of time. The burst size of \( \Phi X174 \) in \( E. coli \) \( C_{syn}^- \) was found to be 80 and latent period was 22 min. Same experiment was performed using \( E. coli \) C as host bacteria and in this case burst size was 100 and latent period 18 min (Fig. 3.01).
Fig. 3. Release of phage from E. coli C (•) and E. coli Csyn- (○) infected with φX174 at m.o.i. = 0.1.

MINUTES AFTER GROWTH

Log (FINAL PFU PER ML)

INITIAL PFU PER ML

0 20 40 60 80 100
Survival Curves of UV Irradiated Free \( \Phi X174 \)

Free \( \Phi X174 \) in tris buffer were irradiated with ultraviolet light for different intervals and at each interval two aliquots were withdrawn. One aliquot was assayed immediately for surviving plaque forming units using \textit{E. coli C (hcr\(^+\))} as host bacteria by double layer agar method, while the other was added to a culture of log phase \textit{E. coli C} in TG medium, allowed to grow at 37\(^\circ\)C for 10 min with aeration, and then assayed as before. The survival of irradiated phage for all doses was observed to be greater as a result of 10 min preincubation in TG medium, the slope of the survival curve in the former case being about 1.50 times of the latter (Fig. 3.02). The mean survival curve of irradiated free \( \Phi X174 \), assayed immediately after irradiation, on either \textit{E. coli C} or \textit{E. coli C\textsubscript{syn-} (hcr\(^-\))} was found to be same, as reported by other workers (Rorsch \textit{et al.}, 1963; Ono & Shimazu, 1966). There was, however, no increase in the survival of irradiated phage when \textit{E. coli C\textsubscript{syn-}} cells were used as hosts and preincubation in TG medium was carried out as in the previous experiment (Fig. 3.02). These results thus show that there was some definite \textit{in vivo} repair of UV irradiated \( \Phi X174 \) within \textit{E. coli C}, but not in \textit{E. coli C\textsubscript{syn-}} cells, due to preincubation in liquid medium. The extent of repair of the UV damage of \( \Phi X174 \) in \textit{E. coli C} was significantly reduced, if preincubation was carried out under condition of
PERCENT SURVIVAL OF PFU

Fig. 3.02.
Fig. 3.02. Survival of UV irradiated OX174 as obtained by plating them on agar immediately after irradiation on *E. coli C* (○) and *E. coli C_{syn}^-* (Δ), and after 10 min pre-incubated in TG medium in *E. coli C* (●) and in *E. coli C_{syn}^-* (□). (■) refers to survival of irradiated OX174 when pre-incubation in *E. coli C* was carried out as before but in presence of 50μg/ml chloramphenicol.
inhibited protein synthesis i.e. in presence of 50 µg/ml chloramphenicol, there being only about 15% change in the slope of the survival curve (Fig. 3.02).

3.03 c Release of Phage from Cells Infected with Irradiated ØX174 Due to Lysis in Liquid Medium

Log-phage E. coli C, growing in tryptone broth, were centrifuged and washed with starvation buffer and then allowed to starve in the same buffer for 1 hr at 37°C with aeration. The starved cells were then infected with UV irradiated ØX174 (1% survival) at gross m.o.i. of 1.0. Adsorption was allowed for 15 min and then the complexes were separated from unadsorbed phage by centrifugation, diluted 10^3 times in TG medium to prevent second cycle infection during subsequent growth* and incubated at 37°C with aeration. The titer of free phage plus infective centers was assayed from time to time. A parallel experiment was performed with the same irradiated phage but using E. coli Csyn−, grown and starved in the identical manner, as host cells. With the passage of time release of phage from E. coli C

* The total number of bacteria (infected and uninfected) in the growth medium after dilution was about 10^5/ml in all cases and the concentration of phage due to lysis of complexes infected with undamaged phage would probably be of the order of 10^5/ml at the end of one hour. At this low bacterial concentration neither phage adsorption (Fujimura & Kaesberg, 1960) nor increase in phage titer due to second cycle infection (Table 3.01) was possible.
Table 3.01

Evidence for absence of adsorption and 2nd cycle infection at low bacterial concentration.

<table>
<thead>
<tr>
<th>No. of bacteriophages of E. coli C</th>
<th>Initial phage titer</th>
<th>Final phage titer after one hour</th>
<th>Relative increase in titer</th>
<th>T initial/T final</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 10^6/ml</td>
<td>1 x 10^5/ml</td>
<td>3 x 10^6/ml</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2 x 10^5/ml</td>
<td>1 x 10^5/ml</td>
<td>3 x 10^5/ml</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Log phase E. coli C cells were infected with φX174 and incubated for one hour.
Fig. 3.03. Release of phage from *E. coli* C (○, □, ▽, △) and *E. coli* C$_{syn-}$ (▲, ●, ■) infected with UV irradiated φX174 (survival 1%) at gross m.o.i. = 1, when the infected cells were grown in TG medium at 37°C with aeration. (⊗, ⊘) indicate phage release from similarly infected *E. coli* C cells when the complexes exposed for 15 min to 0.02 M KCN in TG medium before being allowed to grow in KCN free medium as before. Data from a number of experiments are shown.
was found to be greater than that from *E. coli* C syn- (Fig. 3.03). After 2 hr of growth total number of plaque formers increased \(10^3\) to \(10^4\) times in *E. coli* C while such increase was only 50- to 70-fold in *E. coli* C syn- (Table 3.03). It is to be emphasized here that burst size and latent period for growth of \(\phi X174\) in these two strains of bacteria, as determined in single-step growth experiments, are of the same order, viz. 100 and 17 min for *E. coli* C and 86 and 22 min for *E. coli* C syn-, respectively; also the rate of adsorption of total phage (irradiated and unirradiated) to *E. coli* C and to *E. coli* C syn-, as measured by transfer of \(P^{32}\) counts from free phage to complexes, was more or less same (Table 3.02).

An approximate \(10^2\)-fold increase of the number of plaque formers in either strain can be accounted for as due to bursts of complexes formed by phage which escaped irradiation. The additional increase of plaque formers in *E. coli* C must, therefore, imply *in vivo* repair of UV irradiation damage sustained by \(\phi X174\). If the burst size of UV irradiated \(\phi X174\), after *in vivo* repair, was same as that of normal phage, the \(10^3\) to \(10^4\)-fold increase of titer from infected complexes - of which only one per cent was initially viable - suggests that 10 to 50 per cent of UV damaged phage could be repaired.

A similar experiment was then carried out using *E. coli* C, starved and infected with irradiated \(\phi X174\) as
Table 3.02

Rates of adsorption of φX174 to E. coli C and E. coli C_syn−.

<table>
<thead>
<tr>
<th>Host cells</th>
<th>32P counts of free phage cpm/ml</th>
<th>32P counts of complexes cpm/ml</th>
<th>% adsorption in 15 min adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli C</td>
<td>3.11 x 10^5</td>
<td>1.86 x 10^5</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>3.34 x 10^5</td>
<td>1.99 x 10^5</td>
<td>64</td>
</tr>
<tr>
<td>E. coli C_syn−</td>
<td>3.12 x 10^5</td>
<td>2.17 x 10^5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>3.48 x 10^5</td>
<td>1.97 x 10^5</td>
<td>56</td>
</tr>
</tbody>
</table>

Log phase bacteria were infected with 32P-labeled φX174 at m.o.i = 1 and allowed 15 min for adsorption, centrifuged to discard non-absorbed phage and suspended in same volume of medium. Radioactive counts of free phage and of complexes after 15 min adsorption were measured with G.M. counter.
Table 3.03

Phage release due to lysis in liquid medium of bacteria infected with UV irradiated $\phi$X174.

<table>
<thead>
<tr>
<th>Nature of the complexes</th>
<th>Experiment number</th>
<th>Logarithm of ratio of final phage titer after 2 hr to initial titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E. \text{coli } C + UV'd \phi X$</td>
<td>A1</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td>3.82</td>
</tr>
<tr>
<td>$E. \text{coli } C_{syn} + UV'd \phi X$</td>
<td>B1</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td>B4</td>
<td>1.74</td>
</tr>
<tr>
<td>$E. \text{coli } C + UV'd \phi X$ pre-exposed to 0.02 M KCN</td>
<td>C1</td>
<td>1.91</td>
</tr>
<tr>
<td>for 15 min</td>
<td>C2</td>
<td>2.26</td>
</tr>
</tbody>
</table>
before, but this time phage-bacteria complexes were initially incubated in TG medium in presence of 0.02 M KCN at 37°C for 15 min. KCN was then removed by centrifugation and the complexes were allowed to grow normally in TG medium at 37°C with aeration. Extra increase in the number of plaque formers was absent now; and the titer was practically same as in the case of E. coli Csyn- of the previous experiment (Fig. 3.03) and slight increase in number (Table 3.03) may be due to greater burst size in E. coli C. This set of experiments, which was repeated many times with almost identical results. Although Denhardt and Cairns (1968) have shown that in φX-infected bacteria, phage DNA synthesis in presence of 0.01 M KCN was possible at a very high multiplicity of infection (about 100), in our experimental condition (0.02 M KCN and low m.o.i) conversion of ssDNA to RF appeared to be inhibited (vide infra, Ono & Shimazu, 1967).

It, therefore, appears that this is an essential step in the in vivo repair of UV irradiation damage of φX174.

3.03 d Fate of 32P-labeled ssDNA of UV Irradiated φX174 Within Host Cells

Starved log phase E. coli C and E. coli Csyn- were suspended in TG medium in absence and in presence of 0.02 M KCN. Each of these four cultures were infected at a gross m.o.i. of 1.0 with 32P-labeled φX174, pre-irradiated with UV to a survival of 0.1%, and then incubated at 37°C for 12 min with aeration. Phage-bacteria complexes were
centrifuged, washed and the DNA samples extracted from them were run on 5 to 20% sucrose density gradient in 1/10 dilute SSC at neutral pH as described in Experimental Methods. It appears from these experiments (Fig. 3.04), that $^{32}$P-label, both in the case of *E. coli* C and *E. coli* C$_{syn}$, sediment as a fairly homogeneous band with 21s peak, like $^{32}$P-labeled DNA isolated from complexes infected with normal unirradiated phage. Thus we may identify this as RF DNA in the form of a double stranded circular molecule with both strands continuous (Pouwels, Jansz, Van Rotterdam & Cohen, 1966; Burton & Sinsheimer, 1965) and conclude that irradiated ssDNA was capable of conversion to RF both within *E. coli* C and *E. coli* C$_{syn}$.

When KCN was present, radioactive DNA from *E. coli* C infected with UV irradiated ØX174, sedimented comparatively slowly as a single band with 14s peak, while that from similarly infected *E. coli* C$_{syn}$- appeared to be heterogeneous and on the whole sedimented at a faster rate almost like the ssDNA from free ØX174. It may therefore, be inferred from these sedimentation patterns that in presence of KCN, UV irradiated ssDNA of ØX174 generally remained single-stranded in both cases; it, however, retained its ring structure inside *E. coli* C$_{syn}$-, but was cleaved to a linear molecule in *E. coli* C. Such an inference was also made by Ono and Shimazu (1967) in their work with bacteriophage ØR.
On the basis of our results we propose that as soon as the ssDNA of \( \Phi X174 \) containing UV damage entered an her\(^+ \) host cell, two possibilities obtained. If the conversion of ssDNA to RF was somewhat delayed – as it probably occurred in semi-solid agar plating, or when the phage-infected bacteria were exposed to 0.02 M KCN – the excision enzyme(s) of her\(^+ \) strain attacked the irradiated circular ssDNA disrupting it into a linear molecule. Its biological activity was thereby lost for ever because only circular molecules are capable of plaque formation (Fiers & Sinsheimer, 1962; Pouwels et al, 1966).

On the other hand when rapid growth was feasible in liquid TG medium at 37\( ^\circ \)C with aeration, ssDNA in spite of its UV damage, had some chance of converting itself into circular RF even within the her\(^+ \) host, escaping prior scission and repair of the damage was then possible. It should, however, be mentioned that when her\(^+ \) cells infected with irradiated phage were initially grown for 10 min in TG medium in presence of chloramphenicol to inhibit protein synthesis and then plated on agar, the extent of repair was greatly reduced (Fig. 3.02). It is thus apparent that the whole process of repair must be strongly influenced by the growth rate and metabolic state of infected cells and hence
highly dependent on experimental conditions. It is quite probable that the mechanisms of \textit{in vivo} repair of UV damage of ssDNA within \textit{hor} \textsuperscript{+} cells is not exactly similar to that proposed for the repair of UV irradiated double-stranded bacterial DNA (Setlow & Carrier, 1964; Boyce & Howard-Flanders, 1964; Pettijohn & Hanawalt, 1964). It may be mentioned from the observation of Davis (1964) that there was only 0.38 dimer per lethal hit in \textit{\phi}X174, which meant that other photoproducts must be responsible for more than half of the lethal effect in \textit{\phi}X174.

The UV irradiation damage on the ssDNA template did not act as a block for the enzyme which mediated the conversion of ssDNA to RF. The \textit{minus} strand of the resultant RF was apparently without any gap, although its plus strand contained UV damage, but whether it had any other abnormality like alkali-labile bonds (cf. Rupp & Howard-Flanders, 1968) has yet to be ascertained. It, of course, remained biologically inactive, unless the inherent UV damage was ultimately repaired, as the experiments with the \textit{hor} \textsuperscript{-} strain, \textit{E. coli} C \text{syn-} clearly revealed.