CHAPTER V: BIOCHEMISTRY OF HIS INDUCED LESIONS
Introduction

Hypertonic shock to bacterial cells adversely affected the growth (Costilow, 1981) which was attributed to the inhibition in active transport of carbohydrates (Roth et al., 1985a). Roth et al. (1985b) demonstrated that membrane components of transport system of E. coli lost their normal function as a result of hypertonic treatment. It has also been reported in case of Bacillus subtilis that DNA and other compounds are released under the high salt treatment conditions (Iijima and Ikeda, 1969). Accumulation of carbohydrates during the growth of E. coli was also found at low water activity (Roller and Anagnostopoulos, 1982). Under the hypotonic conditions, the cells suffered a loss of ribosomes probably due to their increased disintegration in a low magnesium medium (McCarthy, 1962).

The involvement of radiation repair genes has long been suggested in normal metabolism e.g. genetic recombination (Howard-Flanders and Theriot, 1966). Subsequent work also provided the basis for their role in the alleviation of the damages caused by non physiological environmental conditions (Bridges et al., 1969; Ahmad et al., 1978; Bennett et al., 1986). We have also observed the recovery of HIS shocked E. coli cells in the growth supporting medium which could not occur in the radiation and heat sensitive recA mutant (chapter III).
In vitro studies conducted for the DNA exposed to high ionic strength revealed a decrease in the sedimentation rate as well as in the number of supertwists in circular lambda DNA (Bode, 1968; Studier, 1969). Destabilization of DNA double helix (Baba and Kagamoto, 1974) and $B \rightarrow Z$ conformational transition (Cavailles et al., 1984) as a result of prolong exposure to certain high salt treatments have also been reported.

In view of the literature mentioned above it appeared worthwhile to carry out the studies on the DNA and RNA metabolism as well as the possible permeability changes in E. coli under our experimental conditions. We have described in this chapter our results on the DNA and RNA syntheses and degradation during recovery as well as leakage of these molecules during the treatment both in the w.t. and recA strains. An attempt was also made to elucidate the nature of HIS induced lesions in DNA. Therefore, certain immunological, biochemical and genetic techniques were also employed for the in vitro studies.

Materials and Methods

Composition of scintillation fluid:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO</td>
<td>4 gm</td>
</tr>
<tr>
<td>POPOP</td>
<td>10 mg</td>
</tr>
<tr>
<td>Toluene</td>
<td>1 litre</td>
</tr>
</tbody>
</table>
Specification of radioactive chemicals:

- $\text{H}^3$-uridine (specific activity 14000 mCi/mM)
- $\text{C}^{14}$-thymine (specific activity 53.5 mCi/mK)
- $\text{H}^3$-benzo-a-pyrene (specific activity 5300 mCi/mM)

Labelling of the cells: The wild-type (AB1157) and HIS sensitive recA mutant (AB2463) of E.coli K-12 were used for this purpose. The strains were grown exponentially in presence of 0.4 µCi/ml of $\text{C}^{14}$-thymine and 4.0 µCi/ml of $\text{H}^3$-uridine. The labelled cells were then held for 2 h in fresh medium without any of the above precursors in order to chase the label. The culture so obtained was employed for studies on DNA and RNA degradation as well as for leakage experiments.

DNA and RNA syntheses during recovery: The recovery medium (chapter II) containing 0.4 µCi/ml $\text{C}^{14}$-thymine and 4.0 µCi/ml $\text{H}^3$-uridine was used for DNA and RNA syntheses respectively. Prior to incubation in the recovery medium, the labelled exponential culture of wild-type and recA strain was exposed to HIS treatment for 4 h. Aliquots (1.0 ml) were taken out at various time intervals during post treatment incubation in the recovery medium and the cells were sedimented by centrifugation at 3,000 rpm for 5 min. The pellet was thoroughly washed with
cold recovery medium and then it was fixed with 5% TCA for 30 min in cold. The suspension containing the labelled cells was then transferred to the filter discs and warmed completely with the help of a high watt bulb. The dried discs were taken into the vials containing 10 ml of scintillation fluid. The radioactivity was determined on LKB 1215 liquid scintillation counter. The untreated controls were also run simultaneously.

**DNA and RNA degradation during recovery:** The wild-type and **recA** strains of *E. coli* were labelled as described earlier. Culture of the labelled strains was treated with high ionic strength for 4 h. The treated cells were then suspended in the recovery medium devoid of radioactive precursors followed by incubation at 37°C. Aliquots were withdrawn at different time intervals during liquid holding in the recovery medium. The samples were fixed by cold TCA and processed for radioactive counts as described earlier. The untreated controls were also run under the identical conditions.

**DNA and RNA leakage during HIS treatment:** The labelled AB1157 and **recA** strains were also used for leakage studies. Exponential cultures of these strains were given a HIS shock for 6 h. At 0, 2, 4 and 6 h of incubation, 1.0 ml cell suspension was withdrawn from the treatment medium and centrifuged.
0.5 ml of the supernatants was carefully taken out, poured on the filter discs and dried completely as described earlier. The radioactive counts present in the supernatant were determined on the liquid scintillation counter.

**Permeability of cells to \(^3\)H-benzo-a-pyrene (bap):** Inward permeability studies were carried out using the wild-type, AB1157 strain. The exponentially grown cells were centrifuged, washed and incubated in the HIS treatment buffer for a maximum period of 6 h. After incubation of the cell suspension at 37°C for 0, 2, 4 and 6 h, 1.0 ml samples were withdrawn and exposed to \(^3\)H-bap for 10 min. The cell suspensions were then centrifuged and the pellet was washed twice with 0.01% MgSO\(_4\) solution containing 0.05 mM unlabelled bap in order to avoid the leakage of label from the cells.

The treated cells were also held in the recovery medium for 2 h. After various time intervals the cells (1.0 ml) were exposed to \(^3\)H-bap for 10 min during the liquid holding in the recovery medium followed by their thorough washing to remove the excess amount of the label.

It is known that incubation of bacteria with EDTA enhances the permeability (Leive, 1965). Therefore, for comparison with HIS treatment the bacteria were also held for 2 min in distilled water containing 2x10\(^{-4}\)M EDTA. The reaction was terminated by
0.01M MgSO₄. EDTA treated cells were also similarly exposed to ^H-bap and were processed for β counts as described above.

Transformation of E.coli cells with pBR322 and pUC8 plasmid DNA:

Preparation of competent cells. An overnight culture of HB101 was centrifuged at 4°C at 5,000 rpm for 10 min. The supernatant was discarded and the bacterial pellet was resuspended in equal volume of normal and treatment buffers separately. The tubes were incubated at 37°C for 6 h. The cells were again centrifuged at 5,000 rpm for 10 min and the bacterial pellet was resuspended in 10 ml of ice cold 0.1M MgSO₄ and kept in cold for 10 min. The cells were again centrifuged in cold and the pellet was finally suspended in 10 ml ice cold 0.1M CaCl₂ and incubated at 0°C for 6 h. The cell suspension was then condensed to 1 ml.

Transformation. For transformation, 0.2 ml of the competent cells were taken in two sterile microfuge tubes and 2-3 µg of untreated and HIS treated pBR322/pUC8 DNA was added to each and incubated at 0°C for 30 min. The cells were given a heat shock at 42°C for 5 min and then again kept at 0°C for 20 min. The cells suspension was then mixed with 1 ml of Luria broth and incubated at 37°C for 1 h. It was then suitably diluted and plated on nutrient agar plates supplemented with either 50 µg ml⁻¹ ampicillin or 50 µg ml⁻¹ ampicillin plus 10 µg ml⁻¹ tetracycline. The antibiotic resistant colonies were scored after 0/N incubation at 37°C.
Immunological studies of H10 treated calf thymus DNA:

Preparation of antigen (Ag). Methylated bovine serum albumin was added to the dialysed and treated calf thymus DNA to prepare a complex. The appearance of turbidity was considered as an index of the formation of complex. The antigen was taken into a small beaker and equal volume of Freund's adjuvant was mixed with gentle shaking. The antigen was thoroughly mixed with the adjuvant by pushing the content in and out of the syringes. This DNA Ag was used for the immunization of the rabbit.

Immunization of rabbit. Preimmune serum was isolated from the blood taken directly from the heart of the animal. After a week, 2 ml of Ag (300 µg) was injected intramuscularly into the rabbit for three consecutive weeks followed by a booster dose of 500 µg DNA Ag. Blood was taken from animal's heart after 2nd, 3rd and booster doses of immunization.

Isolation of serum/antiserum. The blood was kept at 4°C for 3 h and then centrifuged at 2,000 rpm for 10 min. Supernatant (serum/antiserum) was carefully collected in a clean tube and stored in deep freezer in presence of 1% sodium azide.
Cross reactivity of HAbs. The cross reactivity of antibodies raised against the HIS treated DNA was determined with ssDNA, ZDNA and alkali treated (pH 10) DNA employing the enzyme linked immunosorbent assay (ELISA). The method of Voller et al. (1976) was essentially followed.

Hydroxyapatite chromatography (HA). HA chromatography was performed as described by Bernardi (1971) using bed volume of 2.3 cc (column dimensions 3x1 cm). HA chromatography of untreated and treated DNA was carried out as follows. 1 mg of DNA suspended in 0.5 ml of TNE was treated with 1M MgSO$_4$ + 5% NaCl for 6 h at 37°C in the presence of 10% formamide. After 6 h of incubation, the salt was removed by dialysing the sample. Samples containing 1 mg of DNA were loaded on the column and the elution was made with a stepwise gradient of phosphate buffer (pH 7.0). 3.0 ml fractions were collected at the rate of 10 ml/h. DNA eluted in various fractions was determined spectrophotometrically at 260 nm.

BND-cellulose chromatography. A slurry of BND-cellulose was prepared in 0.3M NaT buffer and the fines were removed by decantation. The resin was regenerated following the standard procedure which constitutes the stepwise washing with water (50 ml per 6 gm of resin), 0.3M NaT buffer, 50% formamide in 1M NaT buffer and finally with 0.3M NaT. The washed resin suspended in 0.3M NaT was poured into the column of 1 cm diameter containing glass wool at the bottom so as to achieve a column length of
4 cm. The column was equilibrated overnight with 0.3M NET buffer and 1 mg of native and treated calf thymus DNA was separately loaded. Stepwise elution of DNA with the following buffers in the given order was carried out i.e. 0.3M NET, 1M NET, 1M NET + 50% formamide. Seven fractions of 0.3M NET buffer and ten fractions each of 1M NET and 1M NET + 50% formamide were collected at the rate of 10-12 ml/h.

**Preparation of pBR322 DNA:** _E. coli_ HB101 cells harbouring pBR322 plasmid were harvested from 500 ml of amplified culture and the pellet was suspended in 10 ml of 10% sucrose in 50 mM Tris·HCl, pH 8.0. The cell suspension was then treated with 2 ml of fresh lysozyme solution (10 mg/ml in 0.25M Tris HCl, pH 8.0) plus 8 ml of 0.25M EDTA. The mixture was placed on ice for 10 min. 4 ml of 10% SDS was then gently mixed with a glass rod. Finally, 5M sodium chloride was immediately added so as to reach its final concentration to 1M. The suspension was centrifuged at 30,000 rpm for 30 min to remove the particulate matter. The supernatant was then treated with RNase (20 µg/ml) for 2 h at 37°C. The suspension was then extracted twice with phenol chloroform mixture (1:1) and once with chloroform. After each extraction the aqueous phase was transferred to a clean tube. The extract was supplemented with two volumes of ethanol and kept at 20°C for 24 h. The DNA was then recovered by spinning at 15,000 rpm for 20 min at 4°C. The pellet was washed with 70%
ethanol at room temperature and dried in a dessicator. The purified DNA was finally dissolved in 2 ml TE (10 mM Tris HCl, pH 8.0; 0.1 mM EDTA).

**Mini preparation of plasmid DNA:** For mini preparation of plasmid DNA, a slightly modified procedure for the rapid alkaline extraction method of Birnboim and Doly (1980) was followed. Plasmid harbouring HB101 cells were grown O/N at 37°C in nutrient broth in the presence of ampicillin (100 μg ml⁻¹). 3 ml of cells were harvested by centrifugation in eppendorf tubes for 30 sec. The bacterial pellet was suspended and incubated for 30 min at 0°C in 68 μl of lysis solution consisting of 2 ml 50% glucose, 0.2 ml 0.5M EDTA, 0.25 ml 1M Tris HCl, pH 8.0, 20 mg lysozyme and 7.55 ml water. Thereafter, 136 μl alkaline SDS (0.4 ml 25% SDS, 2 ml 1N NaOH and 7.6 ml water) was added and the suspension was incubated for 5 min at 0°C. After centrifugation, the volume of supernatant was measured and half volume of high salt solution was added. Centrifugation for 15 min was performed in cold and the plasmid DNA in the supernatant was precipitated by adding one volume of ethanol and incubating at -70°C for 2 h. The DNA was collected by centrifugation, washed twice with ethanol and dissolved in 50 μl TE buffer.
Restriction analysis of HIS treated DNA: The restriction buffer of EcoRI restriction endonuclease contained 100 mM NaCl, 50 mM Tris HCl (pH 7.5), 10 mM MgCl₂ and 1 mM 2-mercaptoethanol. The 5X restriction buffer for HincII contained 300 mM NaCl, 50 mM Tris HCl (pH 7.5), 50 mM MgCl₂ and 5 mM dithiothreitol.

Restriction digestion was carried out by the addition of 10 units of restriction enzymes in a total reaction mixture of 20 µl containing 1 µg of lambda DNA. After incubation at 37°C for 2 h, the reaction was terminated by immersing the tubes in water bath maintained at 70°C. After 15 min, tubes were taken out of the bath and kept at room temperature for 5 min and 0.02% bromophenol blue was added.

The DNA was treated with 1M MgSO₄ + 5% NaCl for 6 h and formamide (8%) was added to stabilize any structural change 3 h prior to the completion of the treatment. Control was also run under the identical conditions.

Agarose gel electrophoresis. The λ digests (20 µl along with glycerol dye mixture (50% glycerol, 0.25% bromophenol blue) were applied on the slots. Vertical slab gel electrophoresis was carried out using 0.8% agarose. The slab gel was preelectrophoresed at 60mA for 30 min and the normal run was performed at 10 mA in electrophoresis buffer (89 mM Tris, 2.5 mM EDTA and 89 mM boric acid) for 7-9 h. The DNA bands were stained with ethidium bromide
and fluorescent profile was photographed by UV illumination through fotodyne UV 300 transilluminator.

Results

DNA synthesis during recovery: DNA synthesis was investigated in the wild-type and recA strains and the uptake of $^{14}$C-thymine was followed (Fig. 1). The untreated w.t. cells exhibited an enhanced level of $^{14}$C-thymine uptake during the 2 h post treatment incubation in the recovery medium. In the treated w.t. strain, the incorporation of radioprecursor was significantly inhibited as compared to the untreated control. The treated recA mutant, on the other hand, incorporated an insignificant amount of DNA precursor. This weak incorporation of the label is consistent with the poor colony forming ability of this mutant. However, the untreated recA mutant incorporated the label to an appreciable extent but considerably less than that of untreated w.t. strain.

DNA degradation during recovery: Fig. 2 shows the degradation pattern of DNA in treated and untreated wild-type as well as recA strains. For convenience, the initial counts were normalised to 100%. Both the untreated strains did not exhibit a high degree of DNA degradation, though recA mutant seems to have degraded the DNA
to an appreciable extent. Treated cells in contrast, degraded their DNA to a remarkable degree. RecA mutant again exhibited more DNA degradation as compared with w.t. strain.

**DNA leakage during HIS treatment:** The leakage of DNA was investigated both in w.t. as well as in the HIS sensitive recA mutant. The results are shown in Fig. 3. The untreated cells of both strains did not release the labelled DNA to a significant extent during the HIS treatment for 6 h. However, the HIS exposed cells released an appreciable amount of label. RecA mutant released approximately 5% of its total count while the w.t. exhibited a lower degree of leakage i.e. 3% of its total DNA count.

**RNA synthesis during recovery:** The incorporation of $^3$H-uridine was measured for this purpose. The results are shown in Fig. 4. Both the w.t. and recA strains exhibited an increasing degree of uridine incorporation up to 2 h of incubation in the recovery medium. However, HIS treatment drastically inhibited the RNA synthesis in these strains as compared to their untreated counterparts. Moreover, the incorporation was found to be inhibited to a zero level in case of recA mutant.

**RNA degradation during recovery:** Fig. 5 shows the degradation pattern of RNA. Both the w.t. and recA strains did not degrade their RNA to an appreciable extent under the normal physiological conditions. HIS treatment, however, seems to have induced a con-
siderable amount of RNA degradation in both the recA mutant and wild-type cells. Moreover, the recA mutant exhibited a significantly higher level of degradation as compared to isogenic wild-type strain.

**RNA leakage during HIS treatment:** Fig. 6 shows the pattern of RNA leakage in E. coli. Both the wild-type strain and recA mutant did not exhibit a significant amount of RNA leakage under the untreated condition. However, as a result of HIS treatment, the w.t. and recA strains released about 4% and 3% of the total labelled material respectively.

**Permeability of cells to $^3$H-benzo-a-pyrene (bap):** The inward permeability to an inert material, bap is shown in Figs. 7, 8. During HIS treatment, AB1157 cells incorporated $^3$H-bap showing an increasing trend as the treatment prolonged up to 4 h (Fig. 7). Furthermore, these cells also incorporated $^3$H-bap during post treatment incubation in recovery medium (Fig. 8). However, the level of bap incorporation in the HIS treated E. coli was much lower than that for EDTA treated cells (data not shown).

**Transformation:** Table 1 incorporates the data of transformation of E. coli by HIS treated DNA. The transformation frequency of 6 h treated cells with untreated pBR322 as well as with pUC8 DNA remained unaltered to a large extent as compared to the un-
treated control. However, the transformability of untreated cells by the HIS treated pBR322 or pUC8 DNA was found to be reduced to a remarkable extent.

Cross reactivity of HAbs: The data on the immunological studies have been given in Table 2. The antibodies (HAbs) raised against the HIS treated calf thymus DNA were used for the determination of cross reactivity with ZDNA, ssDNA and pH 10 treated DNA. HAbs exhibited a sufficiently strong cross reactivity with ZDNA. These antibodies also showed a significant level of cross reaction with ssDNA and pH 10 treated DNA. However, the degree of cross reactivity with these DNA species was much less than that with the ZDNA.

Hydroxyapatite and BND-cellulose chromatography of HIS treated DNA: There was no significant difference in the elution profiles of the HIS treated and untreated calf thymus DNA (data not shown).

Restriction analysis: Treated DNA exhibited altered digestion pattern with EcoRI and HincII as compared to their respective control. An extra band was noticed with EcoRI digest of HIS treated DNA between the first and second band (Fig. 9), whereas several bands were obtained with HincII (data not shown). The length of the extra band with EcoRI digestion was calculated out to be 9.3 kilobase.
Discussion

Adverse effects in the growth of microorganisms under the high salt medium are well documented (Costilow, 1981). The leakage of DNA and other compounds in Bacillus subtilis (Iijima and Ikeda, 1969), drastic permeability changes in E. coli (Alphen and Lugtenberg, 1977), the altered transcription of various DNA regions (Blanc et al., 1981) as well as the salt induced in vitro $B \rightarrow Z$ conformational transition in DNA (Cavailles et al., 1984; Taboury and Taillandier, 1984) have been reported by several workers.

HIS treatment in E. coli under our experimental conditions caused a significant inhibition of DNA synthesis. The degree of inhibition was more pronounced in the recA mutant than the w.t. strain. Even after 1 h of liquid holding, there was an insignificant $^{14}$C-thymine incorporation in the recA mutant. Since the w.t. and other bacterial strains recover significantly during liquid holding in recovery medium but recA did not (chapter III), the results of DNA synthesis suggest that inhibition of DNA synthesis may be an important effect of high ionic strength, and recA may be required for the resumption of DNA synthesis in treated bacteria. Inhibition of recovery in the treated bacteria in the presence of nalidixic acid also supports this idea (chapter-III). The effect of HIS treatment on DNA synthesis on treated
bacteria and state of synthesis in recovering bacteria have not been shown in earlier studies.

An increased level of DNA degradation during the recovery as well as an appreciable amount of the leakage of DNA precursors during the HIS treatment serve as an appropriate index for the DNA damage in the hypertonically shocked *E. coli* cells. The untreated w.t. cells and the apparently recovery deficient *recA* mutant did not exhibit a significant level of DNA degradation and leakage (Figs. 2,3) which clearly suggests the involvement of DNA repair and metabolism for this type of injury in the cells. Our alkaline unwinding assay with the DNAs isolated from treated and untreated bacteria also demonstrated the presence of significant amount of single strand breaks as a result of HIS treatment (chapter III). These breaks were repaired upon incubation of bacteria in the recovery medium for 60 min at 37°C. Since we could not observe an appreciable amount of single strand breaks under the in vitro condition, the breaks presumably result from the action of cellular nucleases.

Like DNA synthesis, synthesis of RNA was found to be inhibited in the HIS treated *E. coli* cells. The treatment renders the bacterium incapable of synthesising its RNA, though its increased leakage during the treatment logically directs us for a relatively higher uptake of RNA precursors for the recovery. The inhibition in the RNA synthesis as a result of HIS exposure
suggests an important role of RNA synthesis too, for the recovery of treated bacterium. In the previous chapter we have shown that rifampicin, an inhibitor of de novo RNA synthesis, completely abolished the recovery process. Role of RNA metabolism in the hypertonically shocked cells has also been reported earlier (Blanc et al., 1981).

HIS treatment also increases the permeability of cells data which is evident from our outward permeability for intracellular precursors of macromolecules as well as the increased inward permeability for the inert substance, benzo-a-pyrene. Our studies on the transformation of untreated E.coli with HIS treated DNA though suggest the significant alteration in the DNA, the cells however, on treatment regained to a large extent, their normal transformability (Table 1). This finding as well as the results with the invariable leakage of molecules during the treatment as well as during the recovery process directs us to believe that structural deformities in the membrane components of the transport system probably do not play an important role in overall injury of the cells and their subsequent recovery in the liquid medium. Earlier studies carried out with the high salt treatment conditions however, indicated the loss of colony forming ability with a significant release of UV absorbing material (Sato et al., 1972; Roth et al., 1985a,b). These workers attributed this effect to be
the major cause of lethality. Our conclusion is at variance with the above postulation obviously because of our additional studies with the recovering cells.

The lesions in DNA were not observed by the usual biochemical techniques like hydroxyapatite and BND-cellulose chromatography as well as the DNA alkali unwinding assay under \textit{in vitro} conditions probably owing to their relatively lower sensitivities to locate the minor alterations in the DNA double helix. But the DNA alkaline unwinding assay strongly suggested the formation of single strand breaks under the \textit{in vivo} conditions. These results directed us towards the formation of certain kinds of HIS induced lesions presumably amenable to the cellular nucleases. Moreover, a high sensitivity of \textit{polA}\textsuperscript{-} mutant (chapter III) as compared to the wild-type strain further supported the idea of the distortions in DNA induced by HIS treatment obviously reparable by cellular DNA polymerases. We have, therefore, employed certain highly sensitive immunological, biochemical and genetic techniques to find out any minor structural alteration in the DNA molecule.

It is a well known fact that even a minor alteration in the epitopes of antigen significantly affects the binding capacity of the antibodies raised against the same antigen. This is also true with restriction enzymes which are very sensitive molecular scalples to precisely cut the double stranded DNA owing to the precise recognition for their cleavage sites.
Our immunological studies undoubtedly indicated a structural alteration in the DNA molecule apparently resembling the zig zag type of Z conformation as a result of in vitro HIS treatment to the calf thymus DNA. Moreover, an appreciable amount of single strandedness seems to have been produced following the HIS exposure.

Restriction analysis of treated and untreated lambda DNA also suggested quite different structural states of the substrate DNA after the treatment as is evident from the difference in the digestion pattern of treated and untreated DNA molecule. Interestingly enough a fragment of 9.3 kilobase with EcoRI digestion also points out that at least one resistant cleavage site occurred in the treated DNA. On comparison with the nucleotide sequence of lambda genome, this site had a peculiar feature of having high quantity of GC bases in the close vicinity (44940 and 44990th nucleotides) of resistant EcoRI cleavage site. HincII endonuclease, on the other hand, exhibited partial digestion pattern with the treated DNA. This could be due to the formation of numerous lesions throughout the lambda genome since HincII nuclease is known to have several cleavage sites in the DNA. Random distribution of the lesions throughout the length especially in the GC rich regions should render certain cleavage sites resistant to the enzyme action. This could have been probable reason for the partial digestion...
with HincII. Surprisingly the alteration, apparently resembling to ZDNA was sufficient enough to reduce the transformation frequency of *E. coli* (Table 1). Reduction in the frequency of transformation is well documented in several types of damaged DNA like single stranded DNA, intercalated DNA, heated and sonicated DNA (Chakrabarty *et al.*, 1975; Yuqin *et al.*, 1983; Lee and Lim, 1984).

A remarkably higher DNA and RNA degradation in the HIS treated *recA* mutant during the liquid holding recovery seems to have profound effect on the colony forming ability. This mutant under the normal physiological conditions also exhibited an appreciable amount of DNA degradation which clearly suggests the role of *recA* gene in the normal DNA metabolism e.g. genetic recombination (Howard-Flanders and Theriot, 1966). The role of this gene under the non physiological conditions, to cope with the hazardous environmental conditions, seems to be even more justified. This idea gains further support due to the active role played by *recA* in the repair of heat, pH and radiation induced lesions (Witkin, 1976; Ahmad *et al.*, 1978; Walker, 1985; Musarrat, 1987).
Fig. 1 DNA synthesis in wild-type and recA strains during incubation in the recovery medium at 37°C

- Untreated wild-type: ○—○
- HIS treated wild-type: ○—O
- Untreated recA: ▲—▲
- HIS treated recA: △—△
Fig. 2 DNA degradation during incubation in the recovery medium at 37°C

Untreated wild-type
HIS treated wild-type
Untreated recA
HIS treated recA
Fig. 3  Leakage of DNA precursors during HIS treatment at 37°C

Untreated wild-type :  O——O
HIS treated wild-type :  O——O
Untreated recA :  △——△
HIS treated recA :  △——△
Fig. 4  RNA synthesis in the wild-type and recA strains during liquid holding in the recovery medium at 37°C

- Untreated wild-type: ●—●
- HIS treated wild-type: ○—○
- Untreated recA: △—△
- HIS treated recA: △—△
Fig. 5  RNA degradation during incubation in the recovery medium at 37°C

Untreated wild-type : ⬤―⬤
HIS treated wild-type : □―□
Untreated recA : ▲―▲
HIS treated recA : △―△
Fig. 6  Leakage of RNA during HIS treatment at 37°C

Untreated wild-type : ⬤—⬤
HIS treated wild-type : 0—0
Untreated recA : ▲—▲
HIS treated recA : △—△
Fig. 7  Inward permeability of $^3$H-benzo-a- pyrene during HIS treatment to AB1157 (w.t.)
Fig. 8  Inward permeability of $^3$H-benzo-a- 
pyrene during incubation of HIS 
treated wild-type $E$.coli cells in 
the recovery medium at 37$^\circ$C
Fig. 9  Electrophoretic pattern of EcoRI digested DNA
Lane 1  Untreated DNA digested with EcoRI
Lane 2  HIS treated DNA digested with EcoRI
Table 1.  Transformation of \textit{E. coli} (HB101) cells with pBR322 and pUC8 plasmid DNA

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<td>No. of transformants T.F.</td>
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<td>pUC8</td>
<td>500 (6.3\times 10^{-4})</td>
<td>475 (5.9\times 10^{-4}) 7%</td>
<td>184 (2.4\times 10^{-4}) 65%</td>
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<tr>
<td>pBR322</td>
<td>126 (1.6\times 10^{-4})</td>
<td>125 (1.4\times 10^{-4}) 13%</td>
<td>8 (1.3\times 10^{-5}) 92%</td>
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T.F. - Transformation frequency
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<th>-Log serum dilution</th>
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<th>Percent cross reactivity</th>
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</tr>
<tr>
<td></td>
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Statistical mean 92% 82% 72%