CHAPTER IV

INJURY TO DNA AND ITS REPAIR IN *M.RADIOPHILUS* ON EXPOSURE TO RADIATIONS AND RADIOMIMETIC AGENTS

INTRODUCTION

In the previous chapters effects of radiations and chemicals on the viability and certain cellular processes of Micrococcus radiophilus were described. The phenomenal resistance of this bacterium to radiations necessitates a closer look at the types of damage suffered by the cells and their responses to such insults. In the introductory chapter various lines of evidence were cited which lend support to the presumption that the most important lesions formed in the cells after exposure to radiations are located in the DNA material. The question that immediately suggests itself is, whether DNA of highly radioresistant organism like M.radiophilus has a unique chemical composition. Work reported from this laboratory has shown that DNA of M.radiophilus has the G + C content of 82.88% (1). Thus there do not seem to be any striking differences between base composition of M.radiophilus DNA and those of other sensitive bacteria. Another mechanism to explain the radiation resistance would be that the environment around cellular DNA may have a protective effect against radiation attack. It has been shown that cells of M.radiodurans contain relatively high levels of sulfhydryl compounds. Such compounds could mitigate the action of radiolytic products of water thus conferring resistance against gamma radiation exposure. Studies have however shown that the addition of sulfhydryl group

inhibiting chemicals during gamma irradiation did not result in significant lowering in the resistance of M.radiodurans (2). As discussed in Chapter II the peculiar rigid cell wall structure of M.radiophilus cells does not seem to contribute to any significant degree to the high radiation resistance that this organism is endowed with. In investigating the factors underlying the high radiation resistance, it must also be taken into account that the bacterium offers exceptionally high resistance to other DNA damaging agents such as ultraviolet radiation and methyl methanesulfonate. All these considerations underline the possible role of DNA repair processes in the conferment of the attribute of M.radiophilus and other similar microorganisms to sustain intense onslaughts of DNA damaging chemicals. The current status of DNA repair mechanisms in microorganisms has been reviewed in the introductory chapter. We now know a great deal about the operation of the following repair systems: (i) photoreactivation; (ii) excision repair; (iii) post-replication repair. In addition there could be other repair systems operating in the living cells. Thus it has been suggested that in E.coli there exist fast and ultrafast repair systems which rejoin strand breaks efficiently. The production of strand breaks in DNA has been found to be a common manifestation of cellular DNA damage caused by a variety of physical and chemical treatments. The strand breaks could be formed ~~at the~~ either -

directly as in the case of gamma radiation or indirectly after an appropriate processing of the lesion by the enzyme participating in the early steps of DNA repair. The studies to be described in this chapter were devoted to the analysis of strand breaks in M.radiophilus DNA by the action of radiations and other DNA acting agents. The studies also included a detailed examination of the capacity of the cells to rejoin strand breaks.

MATERIALS AND METHODS

Bacterial strain, media and culture conditions M.radiophilus was used in the studies. The growth medium was TGYM, the composition of which has already been described in chapter II.

Radioactive chemicals (Methyl-^{3}H)-thymidine (specific activity 6.5 Ci/mM) was obtained from the Isotope Group of this Research Centre.

Sedimentation analysis of DNA from M.radiophilus cells subjected to various treatments The procedure used for determination of single-strand breaks in DNA of M.radiophilus cells, exposed to radiations or MMS, was essentially that of McGrath and Williams (3) with some modifications. The cellular DNA was labelled by growing the cells in TGYM medium containing 20 μCi/ml, ^{3}H-thymidine for 16 hrs at 30°C. For MMS treatment the cells were suspended in TGYM

medium containing 10 mM MMS and incubated at 30°C for 2 hr. For irradiation experiments cells were suspended in 0.1 M phosphate buffer (pH 7.0) at a density of 10^8 cells/ml. M.radiophilus cells were exposed to various doses of gamma and UV radiations as described earlier. For studying the rejoining of strand breaks the cells were incubated in TGYM medium after respective irradiation or chemical treatments for various times under different conditions.

Spheroplast formation: The cells were suspended in 0.06 M Tris-HCl buffer (pH 7.6) at the density of 10^8 cells/ml. Aliquots of this suspension were incubated with an equal volume of the solution of lysozyme (5 mg/ml in the Tris-HCl buffer) at 37°C for 30 min to convert the cells to spheroplasts.

Lysing of spheroplasts and centrifugation: The spheroplasts (0.5×10^7/0.1 ml) were lysed with 0.2 ml of 0.17 M NaOH on the top of 4.8 ml linear gradients 5 to 20% sucrose (w/v) in 0.1 M NaOH. Complete lysis was observed in 10 min. The gradients were then centrifuged at 30,000 rpm for 90 min at 21°C using an SW65 rotor in a Beckman model L_2-65B ultracentrifuge and fractionated by siphoning on to a Whatman No.3 paper discs (2.5 cm dia.). Generally 30 fractions (each fraction containing 2 drops of approximately 0.16 ml) were obtained per gradient. The paper discs were dried, washed with 10% cold TCA three times followed by

ethanol and acetone and allowed to dry. TCA-insoluble radioactivity in the fractions was determined as described in the previous chapter and expressed as per cent of total radioactivity recovered from the gradient.

The number average (M_N) and weight average (M_W) molecular weights were calculated using the formula (4),

$$M_N = \frac{C_i}{C_i/M_i}$$

$$M_W = \frac{C_i M_i}{C_i}$$

$$M_i = \left[\left(\frac{7.1 \times 10^{10} \times d_i}{W^2 t} \right) \times \frac{1}{0.0525} \right]^{2.5}$$

where C_i = the radioactive counts for each fraction,

d_i = distance of each fraction in cm from the meniscus,

W = rpm,

and t = time in hr.

The number of single-strand breaks (N) in the DNA for 10^8 daltons were calculated by the formula

$$N = \left(\frac{1}{MNR} - \frac{1}{MNO} \right) \times 10^8$$

where MNR = M_N for DNA from irradiated or MMS treated cells and

MNO = M_N for DNA from control unirradiated cells.

For calculation of M_N from the data on sedimentation profiles, the first 13 - 18 fractions from the bottom were excluded as these generally contained DNA fragments with anomalous sedimentation behaviour.

RESULTS

Effect of gamma radiation exposure on strand breaks in cellular DNA in M.radiophilus

In the first series of experiments strand breaks production in DNA of M.radiophilus cells after exposure to gamma radiation were studied. The determinations were made on the basis of sedimentation behaviour of cellular DNA (pre-labelled with radioactive thymidine) by alkaline sucrose density gradient centrifugation. The method of McGrath and William (3) has been generally used for the determination of strand breaks in irradiated cells. In the case of M.radiophilus the procedure encountered several difficulties mainly due to the rigidity of the cell-wall structure. The concentration of lysozyme to be added to cell suspension in Tris-HCl buffer was hence increased to 5 mg/ml. Since sucrose density gradient sedimentation of cellular DNA is carried out under alkaline conditions, the method determines both single-, double-strand breaks, as also alkali labile lesions formed in the DNA. The sedimentation profiles of DNA from unirradiated cells and from cells irradiated at 200, 500 and 750 krad are illustrated in Fig.1. For calculations

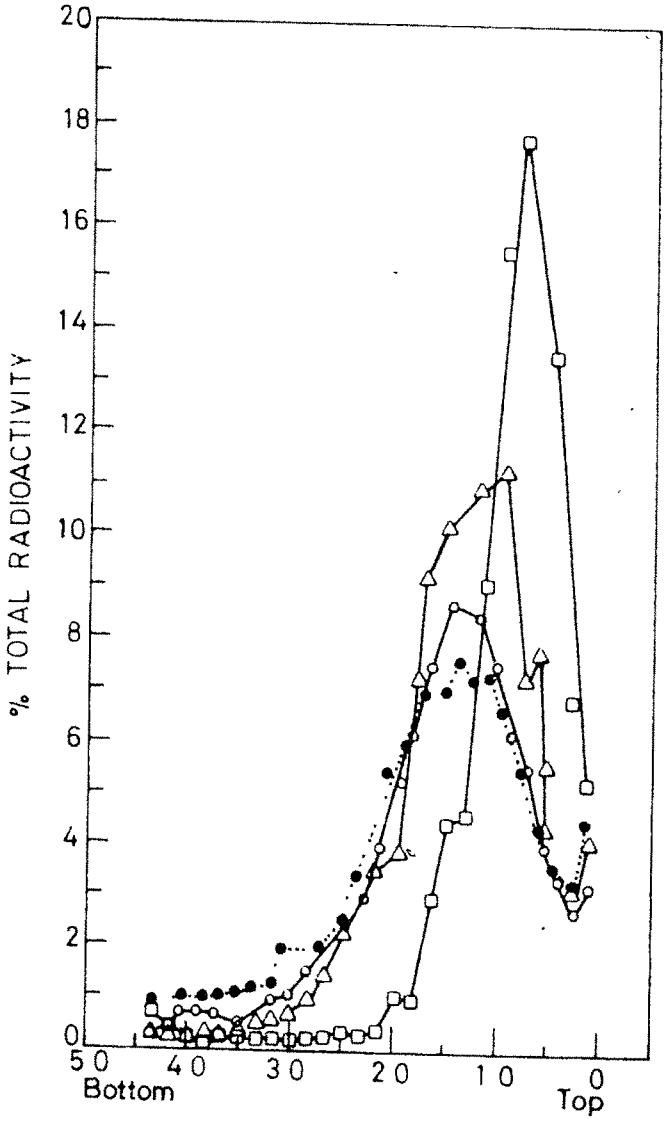

Figure 1. DISTANCE (cm) TRAVELLED FROM MINISCUS

Sedimentation profiles of DNA from M.radiophilus cells, exposed to different doses of gamma radiation, in alkaline sucrose density gradients. In a typical experiment spheroplasts prepared from 5 x 10^6 cells (equivalent to 20,000 cpm ^{3}H-thymidine-DNA radioactivity) were layered on a gradient (4.8 ml), the activity recovered from the fractions (2 drops - 0.16 ml per fraction) is 80%. (..●..), unirradiated; (-o-), 200 krad; (-△-), 500 krad; (-□-), 750 krad.

of strand breaks produced in DNA, number average molecular weights of cellular DNA were determined. To eliminate artefacts in these determinations the bottom fractions were excluded for the calculation of M_N (see Materials and Methods). The strand breaks were calculated from M_Ns of unirradiated and irradiated cells as described in materials and methods. Table I gives the data on M_N and strand breaks as calculated from the profiles illustrated in Fig.1 and in subsequent figures. It will be seen from this table that gamma irradiation of cells at 200 krad (within the shoulder region) and 500 krad (D_{10} value) resulted in only a very few strand breaks. It may be mentioned that these doses are very high compared to D_{10} values of E.coli and other bacteria (the range of 10 - 30 krad). It may be pertinent to mention here that the strand breaks produced in DNA of an E.coli strain are on an average 1.1 break per 10^8 daltons of cellular DNA at the dose of 20 krad gamma irradiation (20% survival) (5). The absence of appreciable number of strand breaks is itself indicative of a highly efficient repair system operating in M.radiophilus. Alternatively it is possible that the cellular environment around DNA may not be conducive to production of strand breaks and alkali-labile lesions in M.radiophilus DNA subjected to gamma radiation. At 750 krad, when the survival of M.radiophilus is brought down to 0.05%, the number of strand breaks formed per 10^8 daltons DNA is 3.66 and at 1000 krad, this value is 11.85. Thus it would appear that even at lethal

Table I

M_W AND M_N OF DNA OF M.RADIOPHILUS CELLS AFTER EXPOSURE TO GAMMA RADIATION AND AFTER POST-IRRADIATION INCUBATION UNDER VARIOUS CONDITIONS

	$M_W \times 10^7$	$M_N \times 10^7$	Strand breaks per 10^8 daltons DNA
0 krad	10.790	3.820	-
200 krad	10.860	3.380	0.34
500 krad	10.300	3.296	0.39
750 krad	3.110	1.582	3.66
1000 krad	2.656	0.843	11.85
200 krad + EDTA	3.258	1.332	4.71
200 krad + EDTA, 10 min incubation in TGYM	5.000	2.555	1.28
200 krad + EDTA, 30 min incubation in TGYM	9.520	3.283	0.41
200 krad + EDTA, 120 min incubation in TGYM + chloramphenicol	10.800	3.450	0.26

The values of M_W, M_N and strand breaks have been calculated from the sedimentation profiles of DNA illustrated in Fig. 1 - 4.

doses the strand breaks production is far below from what can be expected from our experience with E.coli and other bacteria.

Strand break production in M.radiophilus cells exposed to gamma radiation in presence of EDTA

As described in the introductory chapter there have been reports to suggest that bacterial cells may be endowed with fast repair systems catalyzing the rejoining of strand breaks produced in cellular DNA in a brief post-irradiation incubation time of 1 to 2 min (6). In E.coli presence of EDTA during irradiation seems to be inhibitory to such repair, resulting in greatly enhanced number of strand breaks (7). Experiments were conducted to see if EDTA added during irradiation likewise leads to greater number of strand breaks in DNA in M.radiophilus by inhibiting fast repair systems. Results of these experiments are illustrated in Fig.2 and the number of strand breaks are shown in Table I. EDTA added during irradiation significantly increased strand breaks produced. Of the EDTA concentrations examined, 25 mM resulted in the optimum effect. At this concentration the number of strand breaks produced at 200 krad gamma radiation was $4.71/10^8$ daltons DNA. Even this value of strand break formation is much smaller than the corresponding value with E.coli irradiated in the presence of EDTA. It may be noted that at 750 krad (0.05% survival) the strand breaks produced are $3.66/10^8$ daltons DNA. As reported in Chapter II

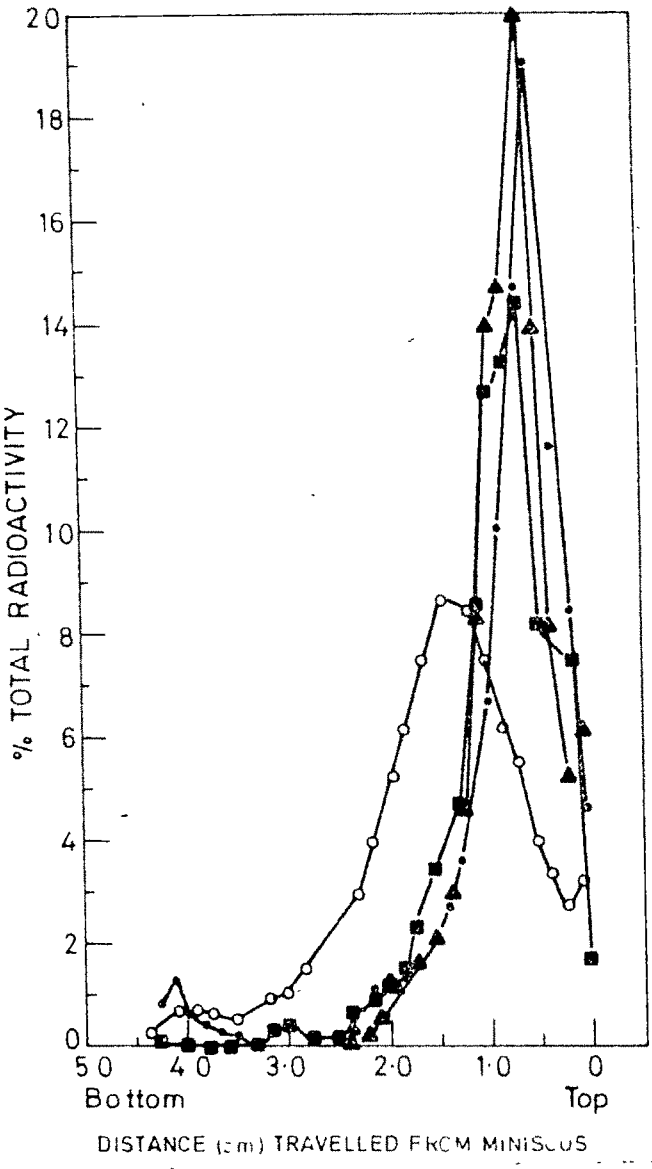

Figure 2. Sedimentation profiles in alkaline sucrose density gradients of DNA from M.radiophilus cells irradiated in presence of different concentrations of EDTA. (-o-), 200 krad; (-●-), 200 krad (25 mM EDTA); (-▲-), 200 krad (50 mM EDTA); (-■-), 200 krad (100 mM EDTA).

irradiation of M.radiophilus at 200 krad, the presence of 25 mM EDTA affected survival only to a small extent with some reduction in the shoulder region. This was due to the fact that EDTA was not present in the plating medium used for viable counts. It is possible therefore that the strand breaks produced in the presence of EDTA may have been repaired after the removal of the chelating agent. Also these results would imply that the nature of strand breaks produced by the lethal dose of 750 krad may be qualitatively different from those produced by the sub-lethal dose of 200 krad in the presence of EDTA as sensitizer.

Repair of strand breaks in cells exposed to gamma radiation in the presence of EDTA

These considerations prompted experiments on the capacity of the cells to repair strand breaks produced by 200 krad in the presence of EDTA. The results of such an experiment are presented in Fig. 3 and the strand break data in Table I. As seen in the Table the strand breaks were efficiently repaired within 10 min of post-irradiation incubation in TGYM broth and by the end of 30 min incubation most of the breaks were rejoined.

It is known that the slow repair of DNA in E.coli may require de novo synthesis of proteins (8). This repair is essentially excision repair which may or may not include inducible-repair. Experiments were conducted to examine

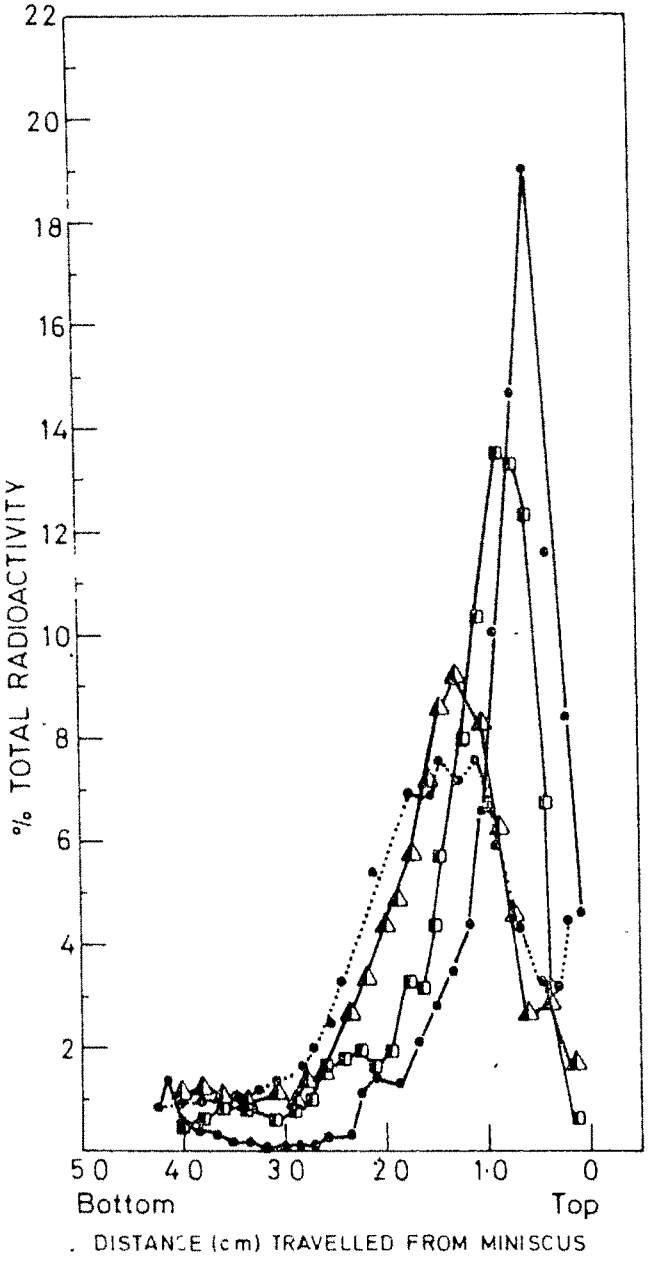

Figure 3. Sedimentation profiles in alkaline sucrose density gradients of DNA from M.radiophilus cells exposed to 200 krad in presence of EDTA followed by post-irradiation incubation in TGYM broth. (·····●·····), unirradiated; (-●-), 200 krad (25 mM EDTA); (-■-), 200 krad (25 mM EDTA): 10 min in TGYM broth; (-▲-), 200 krad (25 mM EDTA): 30 min in TGYM broth.

whether the strand break repairs in M.radiophilus exposed to gamma radiations in the presence of EDTA requires de novo synthesis of proteins. The results are illustrated in Fig. 4 and the strand break data shown in Table I. It will be seen that most of the strand breaks produced were repaired during post-irradiation incubation in TGYM medium in the presence of chloramphenicol. Also the strand breaks could be repaired in phosphate buffer though somewhat at slower rate.

Effect of UV radiation exposure and strand breaks in cellular DNA in M.radiophilus

As shown in Chapter II this organism not only exhibits exceptionally high resistance to gamma radiation but also to UV radiation. It has been well documented that the principal lesion produced in DNA by UV radiation is cyclobutane type dimers of adjacent pyrimidines, predominantly thymine dimers (8). This lesion in DNA can be eliminated by various repair systems (see Chapter I). There are several reports indicating that the production of strand breaks is one of the early steps in the repair of DNA containing pyrimidine dimers (9). Experiments were hence carried out to assess the extent of strand break formation in M.radiophilus DNA after exposure to UV radiation. The sedimentation profiles of DNA from UV irradiated M.radiophilus cells are depicted in Fig. 5 and the strand breaks calculated from these profiles are given in Table II. It will be seen that the nicks formed in DNA after exposure

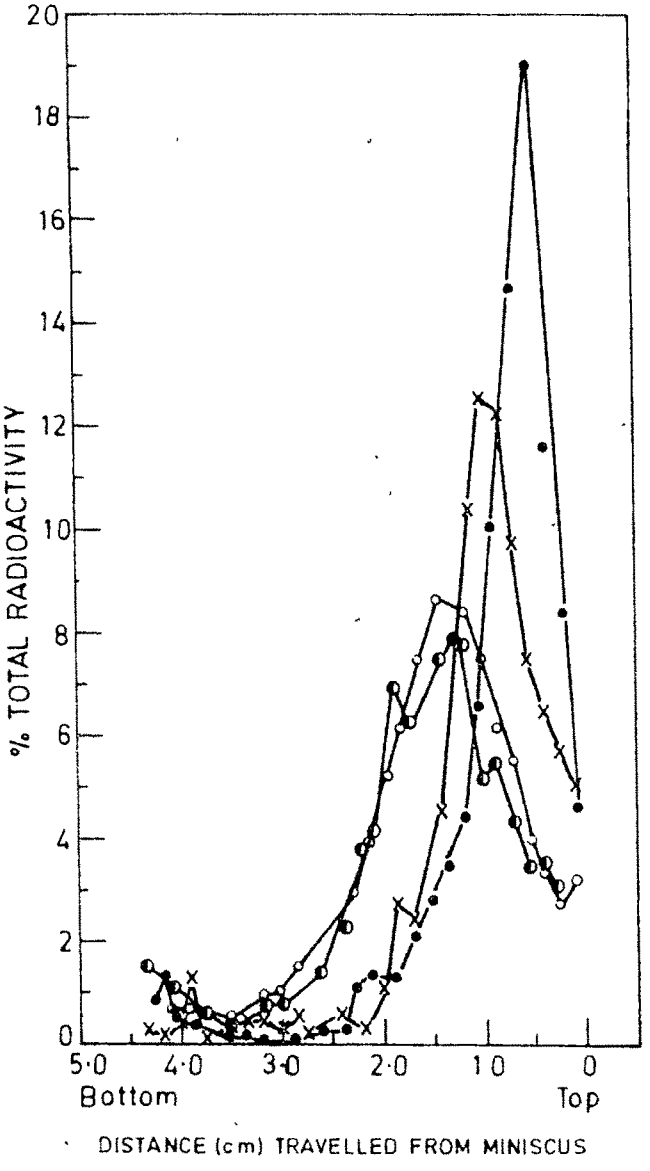

Figure 4. Sedimentation profiles in alkaline sucrose density gradients of DNA from M.radiophilus cells exposed to 200 krad with 25 mM EDTA followed by an incubation under various conditions. (-o-), 200 krad; (-●-), 200 krad (25 mM EDTA); (-x-), 200 krad (25 mM EDTA): 2 hr in phosphate buffer; (-⊙-), 200 krad (25 mM EDTA): 2 hr in TGYM broth plus chloramphenicol.

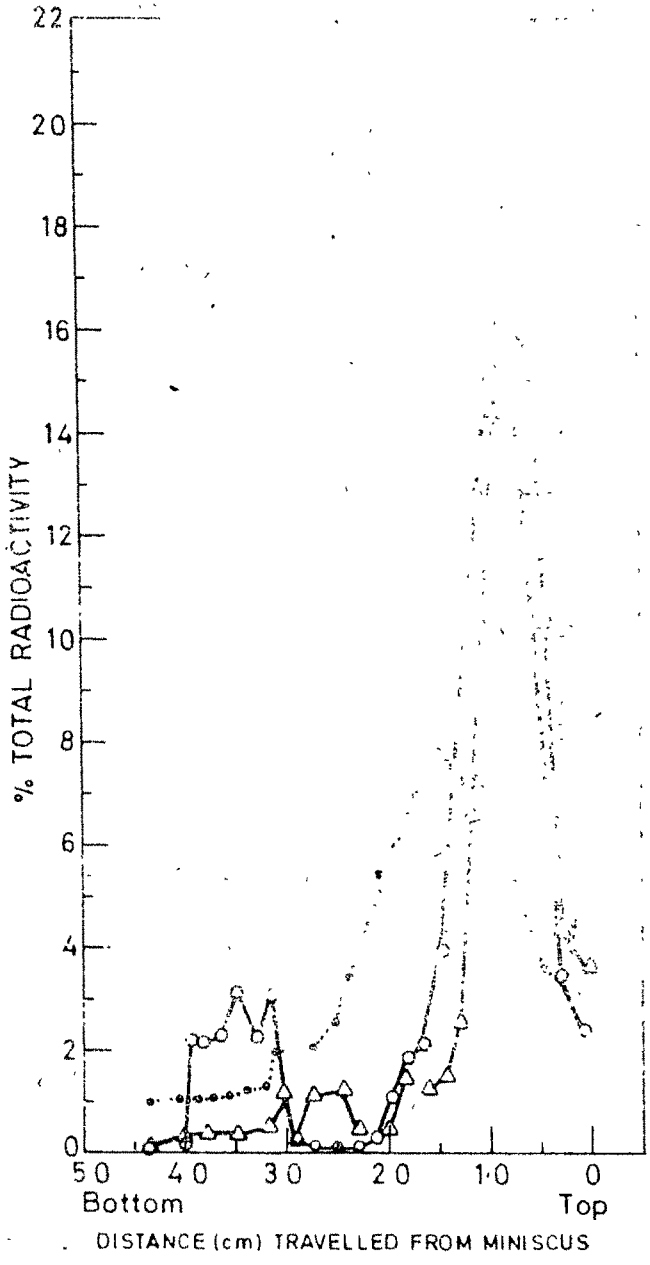

Figure 5. Sedimentation profiles in alkaline sucrose density gradients of DNA from M.radiophilus cells exposed to UV radiation. (..●..), unirradiated; (-o-), 630 J/m^2; (-△-), 1260 J/m^2.

Table II

M_W AND M_N OF DNA OF M.RADIOPHILUS CELLS EXPOSED TO UV-RADIATION AND AFTER POST-IRRADIATION INCUBATION UNDER VARIOUS CONDITIONS

	$M_W \times 10^7$	$M_N \times 10^7$	Strand breaks per 10^8 daltons DNA
Unirradiated	10.780	3.820	-
630 J/m^2	3.477	1.947	2.46
1260 J/m^2	2.210	1.318	4.71
630 J/m^2, 120 min incubation in TGYM	4.13	1.603	3.40
630 J/m^2, 120 min incubation in phosphate buffer	4.224	1.321	4.71

Values for M_W, M_N and strand breaks have been calculated from the sedimentation profiles illustrated in Fig. 5 & 6.

to 630 J/m^2, a dose lying in the shoulder region of the survival curve, are much higher than those produced at 200 krad of gamma radiation (which also is a dose in the shoulder region) and 500 krad of gamma radiation (D_{10} dose). At 1260 J/m^2 (near about D_{10} value of UV radiation survival curve) the strand break production is greater. It may however be mentioned that the strand break production induced by UV-irradiation of M.radiophilus is still much less compared to that produced in E.coli at equivalent doses (9). At the exceptionally high UV radiation doses (in comparison with sublethal and lethal doses for E.coli cells) used for M.radiophilus in the present study it could be expected that the production of pyrimidine dimers could be much higher and consequently the number of strand breaks formed could be also very high. It is implicit in this presumption that the early enzymes of excision repair (including dimers-recognising enzymes) stoichiometrically and efficiently negotiate with pyrimidine dimers to convert them to strand breaks. The failure to score so many strand breaks could therefore be the consequence of highly efficient enzymes responsible for rejoining strand breaks. (9 A)

An experiment was carried out to see if the strand breaks formed in DNA after UV exposure could be repaired during post-irradiation incubation. As seen in the Fig. 6 and Table II post-irradiation incubation in TGYM medium and buffer results in increased number of

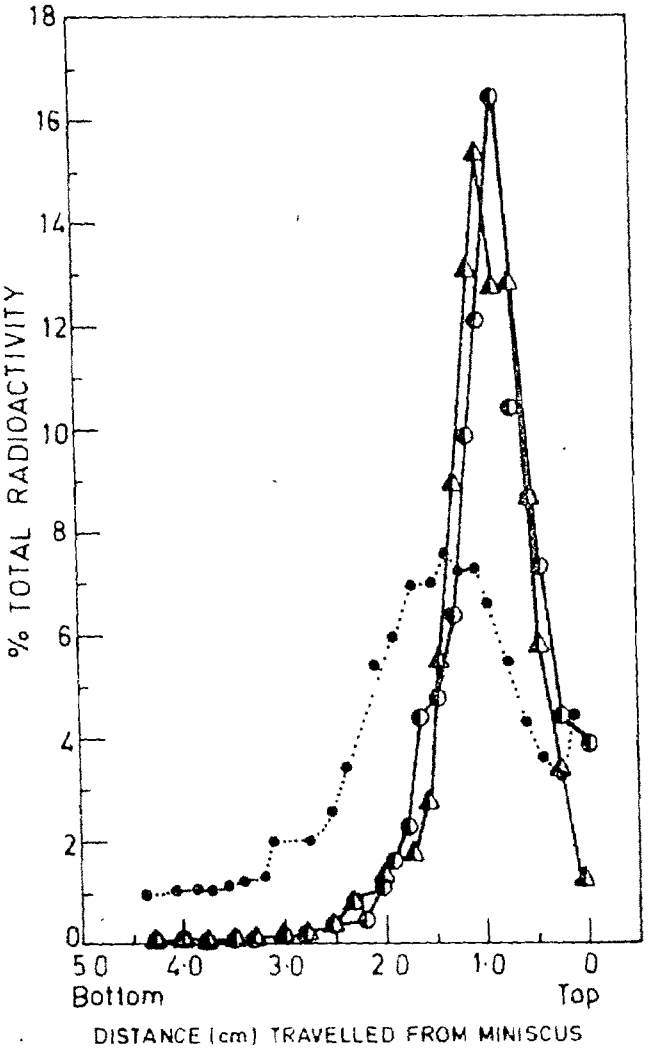

Figure 6. Sedimentation profiles in alkaline sucrose density gradients of DNA from M.radiophilus cells exposed to UV radiation and incubated in phosphate buffer or TGYM broth. (---●---), unirradiated; (-▲-), 630 J/m^2: 2 hr in phosphate buffer; (-◐-), 630 J/m^2: 2 hr in TGYM broth.

strand breaks. The increase in the number of nicks is more in incubation in phosphate buffer compared to that in TGYM medium. Since the dose given in this case is non-lethal it would imply that the observed cuts in cellular DNA at 2 hr post-irradiation incubation could have been repaired eventually. In other words the increased breaks can be considered as a manifestation of a repair process, presumably slow repair in the case of TGYM medium incubation and liquid holding recovery in the case of buffer incubation. Although the question of liquid holding recovery does not arise for the non-lethal dose of 630 J/m^2, other studies have indicated that liquid holding recovery does occur in the case of M.radiophilus cells exposed to lethal doses around 1260 J/m^2 (results not shown). It is also interesting to note that unlike gamma radiation exposure, UV radiation exposure results in significant induction of DNA degradation even at 630 J/m^2 as shown in Chapter III.

Formation of strand breaks in DNA of M.radiophilus cells upon treatment with methyl methane sulfonate

As reported in previous chapters M.radiophilus was also found to be highly resistant to the treatment of MMS. MMS is a chemical mutagen and its effect is expected to arise from its action on cellular DNA. The resistance of bacterial cells to MMS can simply be explained as the result of permeability barrier of the cells. It was hence of interest to ascertain whether the chemical at the concentration lying

in the shoulder region has any effect at all on cellular DNA. The results on the sedimentation behaviour of M. radiophilus DNA in alkaline sucrose density gradient are shown in Fig. 7. The results clearly indicate that MMS at non-lethal concentration does cause strand cuts. Table III gives the data on the number of strand breaks formed in DNA by MMS treatment. As can be seen the number of strand breaks produced in cellular DNA by MMS treatment are much higher than those produced by equivalent doses of UV and gamma radiations (i.e. the shoulder regions of respective survival curves).

Experiments were also conducted to study the fate of strand breaks produced in M.radiophilus cells during post-treatment incubation in TGYM broth. As seen in Table III almost all the strand breaks produced were repaired during incubation for 4 hr in TGYM broth. It will also be seen that inclusion of chloramphenicol did not inhibit the rejoining of strand nicks.

It appears that the rate of strand break rejoining in M.radiophilus cells treated with MMS may be somewhat slower than that observed in M.radiophilus treated with 200 krads gamma radiation exposure in the presence of EDTA as can be seen in Table I; these data are illustrated in Fig.8.

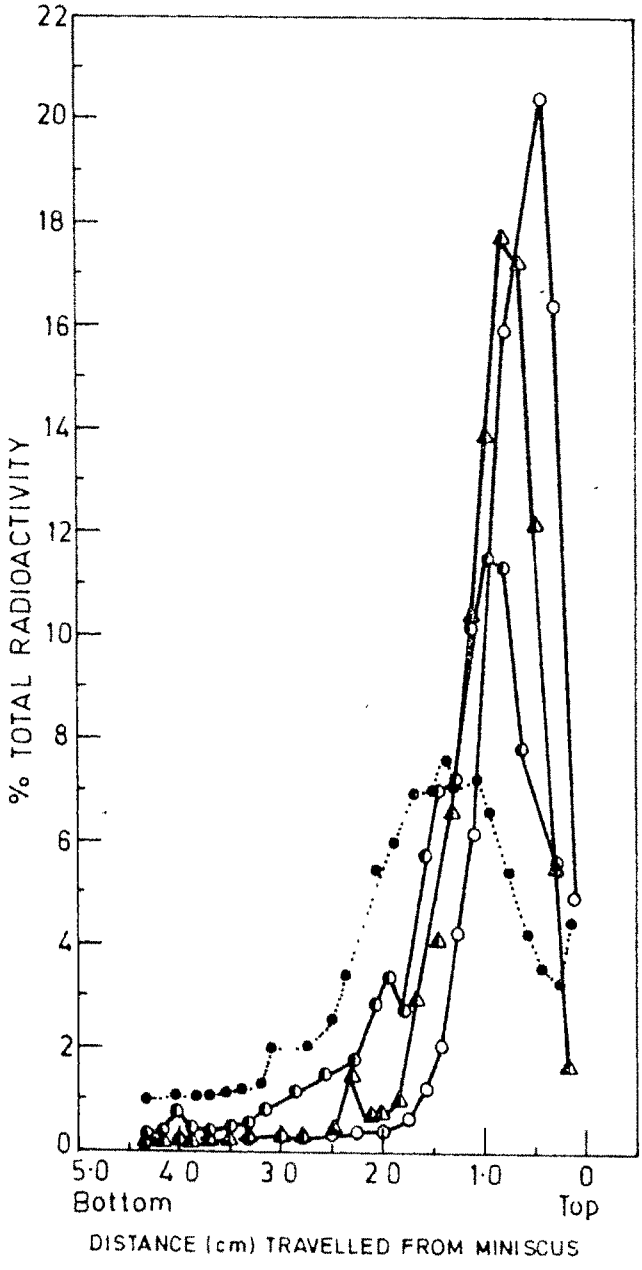

Figure 7. Sedimentation analysis in alkaline sucrose density gradients of DNA from M.radiophilus cells treated with methyl methanesulphonate. (..●..), unirradiated; (-o-), MMS (10 mM); (-▲-), MMS (10 mM): 2 hr in TGYM; (-●-), MMS (10 mM): 2 hr in TGYM plus chloramphenicol.

Table III

M_W AND M_N OF *M.RADIOPHILUS* CELLS AFTER MMS TREATMENT AND AFTER POST-TREATMENT INCUBATION UNDER VARIOUS CONDITIONS

	$M_W \times 10^7$	$M_N \times 10^7$	Strand breaks per 10^8 daltons DNA
Untreated	10.790	3.820	-
2 hr MMS (10 mM)	2.444	1.154	6.07
2 hr MMS (10 mM), 4 hr incubation in TGYM	5.691	1.978	0.83
2 hr MMS (10 mM), 4 hr incubation in TGYM + CM	3.325	1.637	0.88

Values for M_W, M_N and strand breaks have been calculated from sedimentation profiles of DNA depicted in Fig. 7.

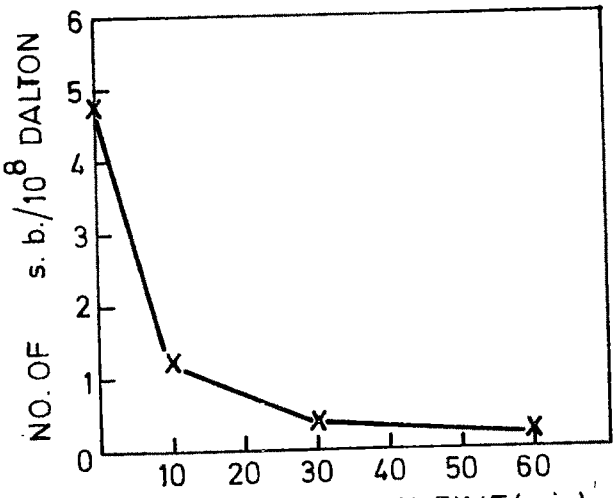

Figure 8

Rate of rejoining of strand breaks in DNA of M.radiophilus cells irradiated at 200 krad in the presence of EDTA, during post-irradiation incubation in TGYM broth at 30°C.

DISCUSSION

The foregoing results clearly show that the inability of various DNA acting physical and chemical agents to injure M.radiophilus cells even at high doses is directly related to the failure of these agents to inflict permanent damage in cellular DNA. Although strand breaks in DNA could be discerned in M.radiophilus cells after exposure to gamma rays, UV radiation and MMS treatment, the number of breaks seem to be very much smaller than what could be expected at such high doses.

It has been reported that single- and double-strand breaks are formed in M.radiodurans by even gamma radiation doses in the non-lethal dose range (11). In contrast the present studies show that no strand breaks are formed in cellular DNA even up to the lethal dose of 500 krad. Also, unlike in the case of M.radiodurans, these doses do not induce any significant degradation of DNA (Chapter III) (11). The operation of fast repair in M.radiophilus is suggested from the experiments on gamma radiation of cells with EDTA. Experiments reported in E.coli indicate that EDTA inhibits fast repair presumably by inhibiting DNA ligase which requires Mg^{2+} as a cofactor (12). Under the conditions of gamma irradiation the presence of EDTA results in enormous increase in single-strand breaks. Although EDTA at the concentration of 25 mM

has indeed increased the strand breaks from a negligible number to 4.7 per 10^8 daltons of DNA, this increase is much smaller judging from the high radiation dose of 200 krad and results with E.coli and other bacteria. It therefore appears that only a very small fraction of gamma radiation-induced lesions (majority of which has been found to be nicks in studies with bacteria) are manifested in M.radiophilus. Conceivably therefore a highly efficient repair of DNA could be in operation in the case of M.radiophilus. The strand breaks formed after irradiation at 200 krad in the presence of EDTA seem to be rapidly rejoined during post-irradiation incubation in growth medium and this repair is not inhibited by chloramphenicol indicating that de novo synthesis of proteins may not be required for this process. Probably therefore this process is a case of short-patch excision repair (see Chapter I). On the other hand it is quite possible that the observed strand break rejoining may not have the intervening excision and resynthesis steps, as has been suggested for EDTA sensitive fast repair in E.coli (7). In other words the observed phenomenon could simply be the case of rejoining of clean breaks (i.e. 3'-OH and 5'-P termini) mediated by DNA ligase (13). Evidence from this laboratory suggests that DNA ligase in E.coli may be located in the cell membrane (14).

It was interesting to observe that unlike gamma irradiation, UV radiation and MMS treatment of M.radiophilus

cells in the non-lethal range resulted in the production of significant number of strand breaks. These results seem to show that the enzymes involved in the recognition of lesions in DNA could be different. In fact there has been suggestive evidence indicating that some of the early enzymes involved in the repair of different types of lesions in DNA could be different (15, 16). This aspect has been discussed in chapter I.

The strand breaks formed in DNA after UV irradiation in the non-lethal dose range are increased in number during post-irradiation incubation in growth medium and during post-irradiation incubation in buffer there is a further increase. The observed increase in the strand breaks in cellular DNA during post-irradiation incubation in growth medium could be the manifestation of both excision and post-replication repair. On the other hand the increase in strand breaks during post-irradiation incubation in buffer could be solely due to excision repair since this condition disallows post-replication repair. The repair albeit seems to be slow since no evidence of strand break rejoining is seen at 2 hr of post-irradiation incubation.

The results on UV irradiation at the non-lethal but high doses (in the E.coli context) bring again the question of non-detectability of damage to cellular DNA.

Other colleagues in this laboratory have analysed the formation of thymine dimers in M.radiophilus cells exposed to UV irradiation and their disappearance during post-irradiation incubation. These results ~~have been~~ are given in Table IV. On the basis of these results the number of thymine dimers formed in M.radiophilus DNA (A + T = 17.12%) (1) can be calculated as 220 per 10^8 dalton of DNA. On the basis of the work in E.coli it may be inferred that very high doses are required for the formation of this number of dimers. It has been suggested that on an average 1 nick can be formed per UV-induced pyrimidine dimer in E.coli presumably by the action of the endonuclease (17, 18). Thus at the dose used in the present study, namely 630 J/m^2, about 279 nicks could have been formed per 10^8 daltons DNA. The results reveal however only a few breaks formed. The non-detectability of strand breaks could be explained as the consequence of extremely efficient strand break rejoining process.

The strand breaks seen after MMS treatment could also be very much lower than expected. MMS, known to methylate DNA at the purine bases and other sites (see Chapter I), could also elicit strand breaks at the rate of 1 strand break per lesion; conceivably the fast strand break rejoining process has suggested for UV radiation may be operative. results discussed above, The strand break rejoining does indeed occur after MMS treatment as shown in Table III.

Table IV

THE LEVELS OF THYMINE DIMERS IN DNA OF M.RADIOPHILUS EXPOSED TO UV IRRADIATION

UV radiation dose	Per cent thymine dimers retained at different post-irradiation incubation time intervals				
(J/m^2)	0 (min)	30 (min)	90 (min)	120 (min)	240 (min)
500	1.8	1.0	0.4	0.1	-
10,000	3.0	1.85	1.0	0.8	0.5

From V.R. Alarmela and N.F. Lewis unpublished data.

This process is also independent of concomitant protein synthesis suggesting that it may be short-patch excision repair and post-replication repair.

The results presented in this chapter have yielded clear evidence to suppose that various physical and chemical treatments do indeed inflict damage on M.radiophilus DNA even at non-lethal doses. These non-lethal doses are however exceptionally high bringing into focus the mechanisms that are responsible for such a unique attribute. Studies described in previous chapters seem to imply that the high resistance of this bacterium may not be due to the rigid cell wall structure.

The organism seems to be endowed with a variety of repair mechanisms as suggested by the present study, for example, fast repair, excision repair, post-replication repair. Since the alkaline sucrose density gradient method analyses both single- and double-strand breaks, it can be inferred that the strand break rejoining seen in cells irradiated with EDTA and treated with MMS could also include repair of double-strand breaks. Repair of double-strand breaks has been demonstrated in another highly radio-resistant microorganism such as M.radiodurans (19). Studies on the strand break repair, especially those given in Fig. 8, seem to show that the organism repairs greater number of strand breaks per unit time than what has been

reported in the case of E.coli. The present studies do not shed any light on the relative role of any of the repair systems mentioned in determining the high radiation resistance that this organism displays.

However the results have emphasised that M.radiophilus could be an ideal system to study various unknown facets of the net-work of DNA repair systems which seem to play a major role in the protection of the living cell to various kinds of physical and chemical attacks on its cellular DNA.

SUMMARY

Studies were conducted to investigate the mechanisms underlying exceptionally high resistance that M.radiophilus displays towards gamma,UV radiations and methyl methane sulfonate. The damage to cellular DNA by these treatments were studied by assessing strand break formatinn by the method based on sedimentation behaviour of DNA in alkaline sucrose density gradient ultracentrifugation. At the doses of 200 krad (shoulder region dose) and 500 krad (D_{10} dose), no strand breaks in DNA were evident. However when cells were irradiated in the presence of EDTA (25 mM), strand breaks were formed at the rate of $4.71/10^8$ daltons of DNA. The breaks so formed could be repaired by post-irradiation incubation in growth medium at 30°C for 30 min. This repair was not inhibited by chloramphenicol. Unlike gamma radiation at 200 krad, UV radiation at the dose of 630 J/m^2 (shoulder region dose) resulted in the formation of strand breaks at the rate of 2.4 breaks/10^8 daltons. DNA. Incubation of the UV-irradiated cells in growth medium or buffer for 2 hr increased the number of strand breaks, the increase was more in the buffer than in the growth medium. Treatment of cells with MMS resulted in the formation of 6.1 strand breaks/10^8 daltons of DNA and these were rejoined during 4 hr post-treatment incubation in growth medium. This repair was not inhibited by chloramphenicol. These results indicate that the number of strand

breaks [redacted] induced in M.radiophilus DNA by very high doses of radiations and DNA acting chemicals are very much small than expected implying either the protection of the DNA from external onslaughts or efficient repair systems. Although the capacity to repair DNA of M.radiophilus seems to be higher than that in E.coli and in other bacteria, it has not been possible from the present results to deduce the relative importance of different types of repair in determining the high resistance exhibited by this bacterium.

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