# CHAPTER II

## SURVIVAL RESPONSES OF *MICROCOCCUS RADIOPHILUS* EXPOSED TO VARIOUS PHYSICAL AND CHEMICAL AGENTS

# INTRODUCTION

There is a wide range of susceptibility to radiations in the microbial world and this has been the topic of intensive investigations for the past several years. Perhaps the major impetus to research in this area was given after the exciting discovery by Anderson in 1956 (1, 2) that a bacterial strain, Micrococcus radiodurans isolated from an irradiated canned meat sample displayed phenomenal resistance to the onslaughts of gamma radiations. Since then several other radio-resistant strains in a variety of food and natural sources have been reported. In this laboratory, a highly radiation-resistant organism was isolated from a sample of a locally available fish, Bombay duck (Harpodon nehereus) by Lewis which is named as Micrococcus radiophilus (3).

There have been several attempts to elucidate the mechanisms underlying the extremely high radiation resistance exhibited by certain micrococci and a gram negative Pseudomonas strain. The DNA content and base composition of radiation resistant bacteria are not particularly unusual (4, 5). The carotenoid pigments present in the radio-resistant micrococci were suggested to play role of radio-protective agents but they were found not to participate in such protection (6). The radioresistant bacteria, in general, have higher sulphydryl content (7) and it has been suggested that this and possibly other entities in the

cellular environment could act as scavengers for radical species produced by gamma irradiation. However it has been observed that although sulphydryl ~~group~~ inhibitors, such as p-hydroxymercuribenzoate, can bring down the resistance of M.radiodurans to some extent, these do not lower the resistance to the level of moderately radio-sensitive bacteria like E.coli.

The radio-resistant micrococci, whose cells are approximately spherical with 1 - 1.6 $\mu$m diameter, occur normally in tetrads, occasionally in pairs or singles. Probably these features especially the tetrad nature of cell population could contribute to the high radiation resistance. However, these characteristics are also shared by some radio-sensitive microorganisms such as M.luteus (8).

An examination of the fine structure of the cells of radioresistant micrococci reveals some unusual features distinctly different from any other bacteria described hitherto. The cell surface comprises 3 - 4 distinct layers, each having a characteristic fine structure. The multilayered structure seems to give the rigidity to the cell wall (9 - 12). It is not known whether similar structural features are displayed by the highly radiation resistant Pseudomonas strain (13). It is tempting to suggest that the unusual features of the cell surface of these microorganisms could be related to the extremely high resistance to radiation. The exceptionally high radiation resistance

could be the result of the presence of some protective agents - the rigid cell wall could be one such agent.

It may be noted that the radio-resistant micro-organisms are also highly resistant to ultraviolet radiations and to DNA damaging chemical agents (14). These considerations are compelling enough to imply that the existance of a highly efficient DNA repair machinery could be wholly or partly responsible for high resistance.

If the radiation resistance is the consequence of the operation of a powerful DNA repair machinery, then it is expected that the cells treated with split radiation doses - with an intervening period of incubation in growth medium - will result in much higher resistance as compared with a single unfractionated dose of equal amount.

The phenomenally high resistance to various chemical and physical agents causing damage to cellular DNA, together with unusual structural features of the cell surfaces of this bacteria, call for a detailed examination on the survival responses of this bacteria to various agents such as freezing and thawing, ultrasonic irradiations which are normally lethal to bacteria.

The present chapter embodies studies aimed at examining responses of M.radiophilus to gamma radiation and other physical and chemical agents. As mentioned earlier

this bacterium was isolated in our laboratory and various studies on physiology and biochemistry of this organism have been carried out.

## MATERIALS AND METHODS

**Bacterial strains** *Micrococcus radiophilus* was isolated by Dr.N.F. Lewis in this laboratory from irradiated Bombay duck (3). *Micrococcus radiodurans* was obtained from Dr. A. Matsuyama, Institute of Physical and Chemical Research, Tokyo, Japan. *E.coli* B/r (ORNL) and *E.coli* Bs-1 were stock strains in our laboratory culture collection.

**Media and cultural conditions** All the bacterial strains were grown in TGYM medium consisting of 0.5% tryptone, 0.1% glucose, 0.3% yeast extract, and 0.005% DL methionine. For determining viable counts, cell suspensions after serial dilutions were plated on the same medium containing 1.5% agar. The plates were incubated at 37°C, overnight for the *E.coli* strains and for four days for the *Micrococcal* strains.

**Chemicals** Methyl methane sulphonate was obtained from K & K Laboratories Inc., N.Y., U.S.A. Lysozyme (Egg white, grade I), ethylenediaminetetraacetic acid (EDTA) and tris-(hydroxymethyl)aminomethane (TRIS) were purchased from Sigma Chemicals Co., St. Louis, U.S.A. All other chemicals were of analytical grade obtained from British Drug House, Bombay, India.

Gamma irradiation — Bacterial cells, harvested from Log phase cultures, were washed and suspended in 0.10 M phosphate buffer (pH 7.0) at a density of about $10^9$ cells/ml in the absence or presence of EDTA (0.025 M). The cell suspensions were exposed to gamma radiations in air at 0°C in a Gamma Cell-220 (Atomic Energy of Canada Ltd., Ottawa, Canada) at a dose rate of 3.4 krad/min. In studies with fractionated doses cells were exposed to different doses of gamma radiation followed by incubation in buffer or TGYM broth for 2 hours before exposing to a second dose of radiation.

Ultraviolet irradiations — Bacterial cells, harvested from log phase cultures were washed and suspended in 0.1 M phosphate buffer (pH 7.0) at a density of $10^8$ cells/ml. A 7 ml aliquot of the suspension layered in a glass petri dish (9 cm diameter) was exposed to UV radiation from a Phillip TUV 15 watt germicidal lamp equipped with a reflector with 95% output at 253.7 nm at a dose rate of 90 $J/m^2/min$ at the distance of 50 cm.

Chemical treatment — Bacterial cells harvested from log phase culture were incubated at 30°C in TGYM broth containing various concentrations of MMS. After 2 hours incubation the cells were washed free off the chemical and their viability determined as described above.

Freezing thawing treatment — Cell suspensions prepared as in case of the gamma radiation treatment were frozen in liquid nitrogen (-195°C). The frozen samples were

thawed at 30°C. The viability of cell cultures which had undergone freezing-thawing cycles at specified times was determined as described earlier.

Temperature shock treatment — Cell suspension of M.radiophilus prepared as for the gamma radiation treatments were incubated at various temperatures for specific time intervals, and viability was determined as described above.

Ultrasonication of bacteria — The cell suspensions of the bacteria were prepared as for the gamma radiation treatment were subjected to ultrasonic disintegration in a Ralsomic Ultrasonic processor model RP250 (Ralsonics India Ltd., Bombay) at the frequency of 22 ± 3 KHZ pulsed at 100 c/sec. The absorbance of the cell suspensions were monitored at 660 nm in a Beckman DU spectrophotometer after specified durations of ultrasonication.

## RESULTS

The effect of gamma-irradiation under oxic conditions on the survival of M.radiophilus cells in phosphate buffer is illustrated in Fig. 1. The log-survival curve exhibited an unusually long shoulder region extending up to 400 krad; after this dose, the kill with increased doses was exponential. The $D_{10}$ value of 500 krad is indicative of exceptionally high radiation resistance of this bacterium.

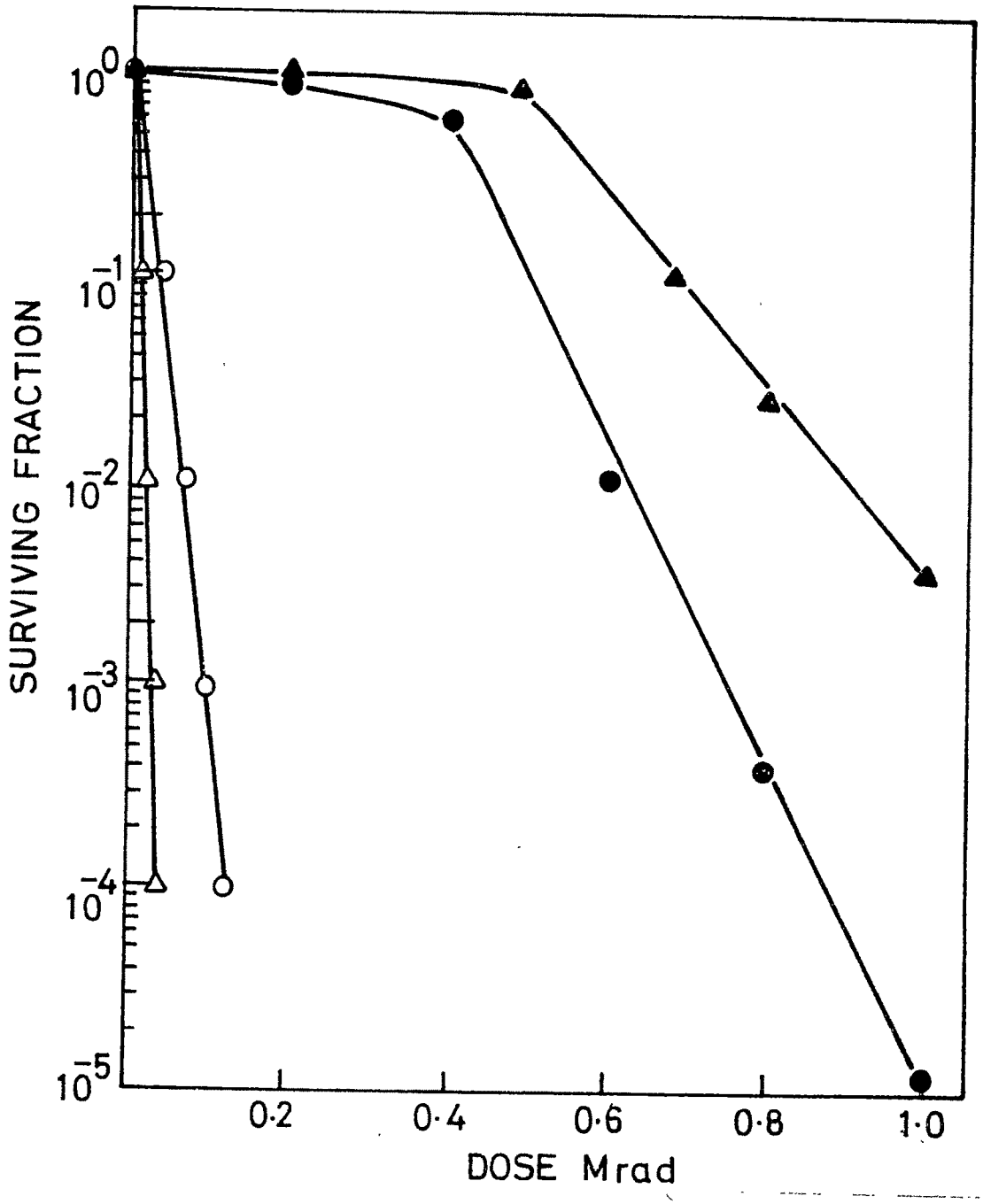


Figure 1. Survival of M.radiophilus, M.radiodurans, E.coli B/r and E.coli Bs-1 exposed to gamma radiation. The bacterial cells were exposed to various doses of gamma radiation in 0.1 M phosphate buffer, pH 7.0, under air at 0°. (-●-), M.radiophilus; (-▲-), M.radiodurans; (-o-), E.coli B/r; (-△-), E.coli Bs-1.

At a dose of 1.0 Mrad, the reduction in survival was of the order of 5 log cycles. $D_{10}$ values of E.coli B/r and of E.coli Bs-1 are very much less being 30 krad and 10 krad respectively. M.radiodurans exhibits somewhat higher resistance than M.radiophilus. The high radiation resistance of M.radiophilus was further enhanced when the cells were irradiated as suspensions in TGYM broth, the shoulder region extending up to as much as 1,6 Mrad (N.F, Lewis, Ph.D. thesis submitted to Bombay University, 1972), due to the protection offered by the medium.

In a separate set of experiments, the response of cells to split doses of gamma-irradiation with an intermediate incubation in TGYM broth or buffer was studied. The results are illustrated in Fig.2. As can be seen in the figure, a single dose of 700 krad resulted in the reduction in the survival up to 0.1%, whereas, if the same dose was split into two fractions of 400 and 300 krad, with an intervening 2 hour incubation in TGYM medium, the survival was about 95%. Similar experiments on the fractional doses of 200 krad - 2 hr TGYM- 300 krad, 600 krad - 2 hr TGYM - 300 krad and 300 krad - 2 hr TGYM - 600 krad (results not shown) indicate that the fractionated doses do not cause any cumulative action. When however doses of 400 krad and 300 krad were given with an intervening 2 hr incubation in phosphate buffer (instead of TGYM broth) the survival was about 35%.

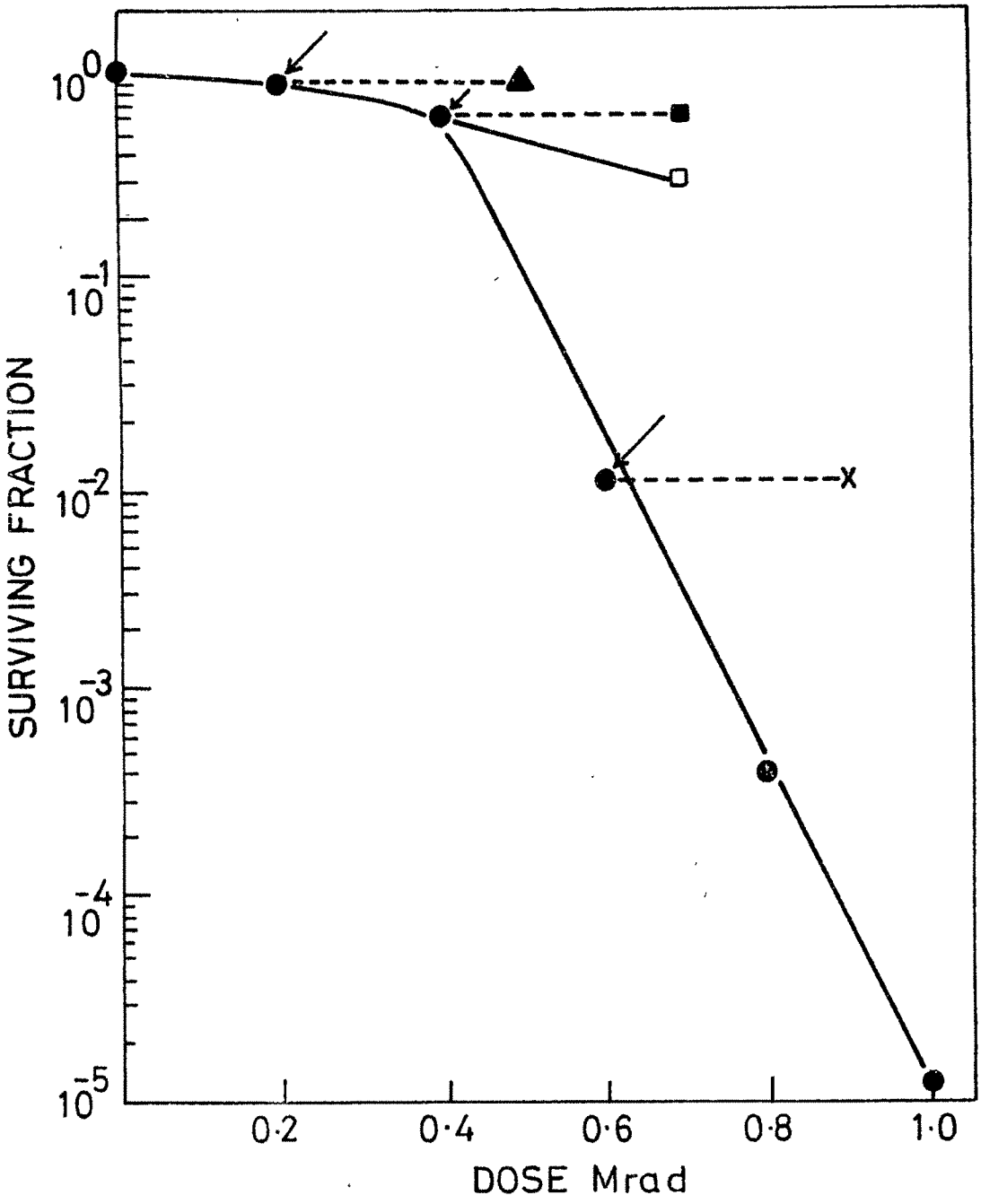


Figure 2. Survival of M.radiophilus cells following exposure to fractionated doses of gamma radiations. Bacterial cells were first exposed to various initial doses of gamma radiation, incubated for 2 hours in buffer or medium and then exposed to a second dose of 300 krad. (-●-), single unfractionated doses; (--▲--), fractionated dose: 200 krad - 2 hr TGYM - 300 krad; (--■--), fractionated dose: 400 krad - 2 hr TGYM - 300 krad; (--x--), fractionated dose: 600 krad - 2 hrs TGYM - 300 krad; (-□-), fractionated dose: 400 krad - 2 hrs buffer - 300 krad.

Studies were conducted to see if radiation resistance can be modified by any treatment of the cells. There are several reports indicating that in E.coli that rejoining of large fraction of single-strand breaks in DNA can be accomplished by mechanisms termed as ultrafast and fast repairs and these are inhibited if EDTA is present along with bacterial cells during irradiation (15). Since efficient DNA repair by cells seems to be an important factor that determines radiation resistance, it was of interest to see if EDTA (by inhibiting fast repair ~~of~~) can increase the susceptibility of cells to gamma irradiation. As seen in Fig. 3, a small but significant sensitization to radiation by EDTA was observed in the shoulder region of the survival curve. The exponential region of the survival curve however was not substantially affected by the presence of EDTA during irradiation.

The presumption that efficient DNA repair may be a factor responsible for high gamma radiation resistance could mean that the organism can be resistant to all the insults which kill the cells by affecting cellular DNA. The action of UV radiation, which is known to affect cellular DNA, was hence ascertained. As seen in Fig. 4, the organism exhibited a very broad shoulder region in the UV radiation survival curve extending up to 900 $J/m^2$ followed by an exponential death phase. Thus M.radiophilus is also resistant to UV radiation. In other studies, it was found that

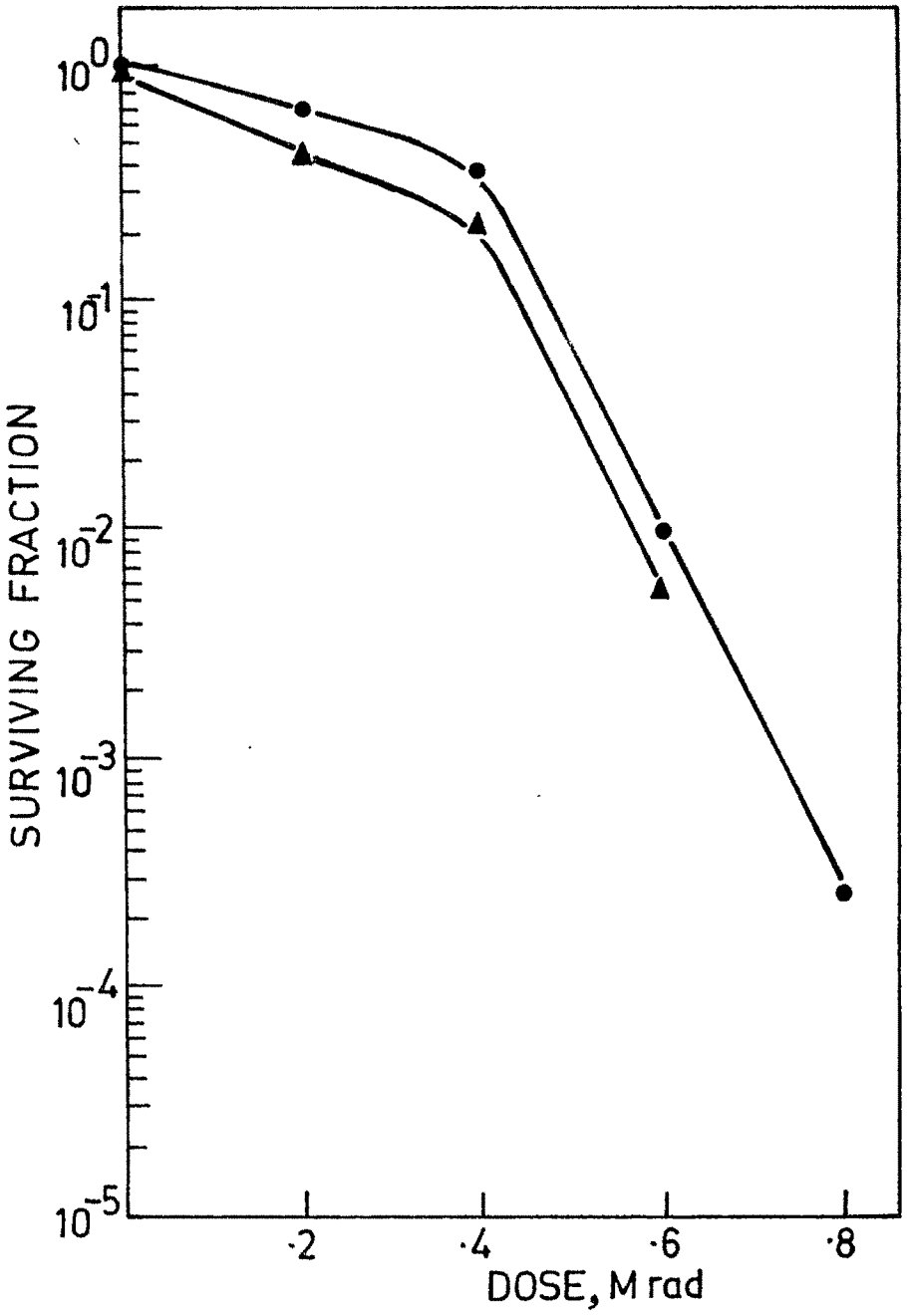


Figure 3. Survival of M.radiophilus exposed to gamma radiation in absence and presence of EDTA (25 mM). (-●-), -EDTA; (-▲-), +EDTA (25 mM).

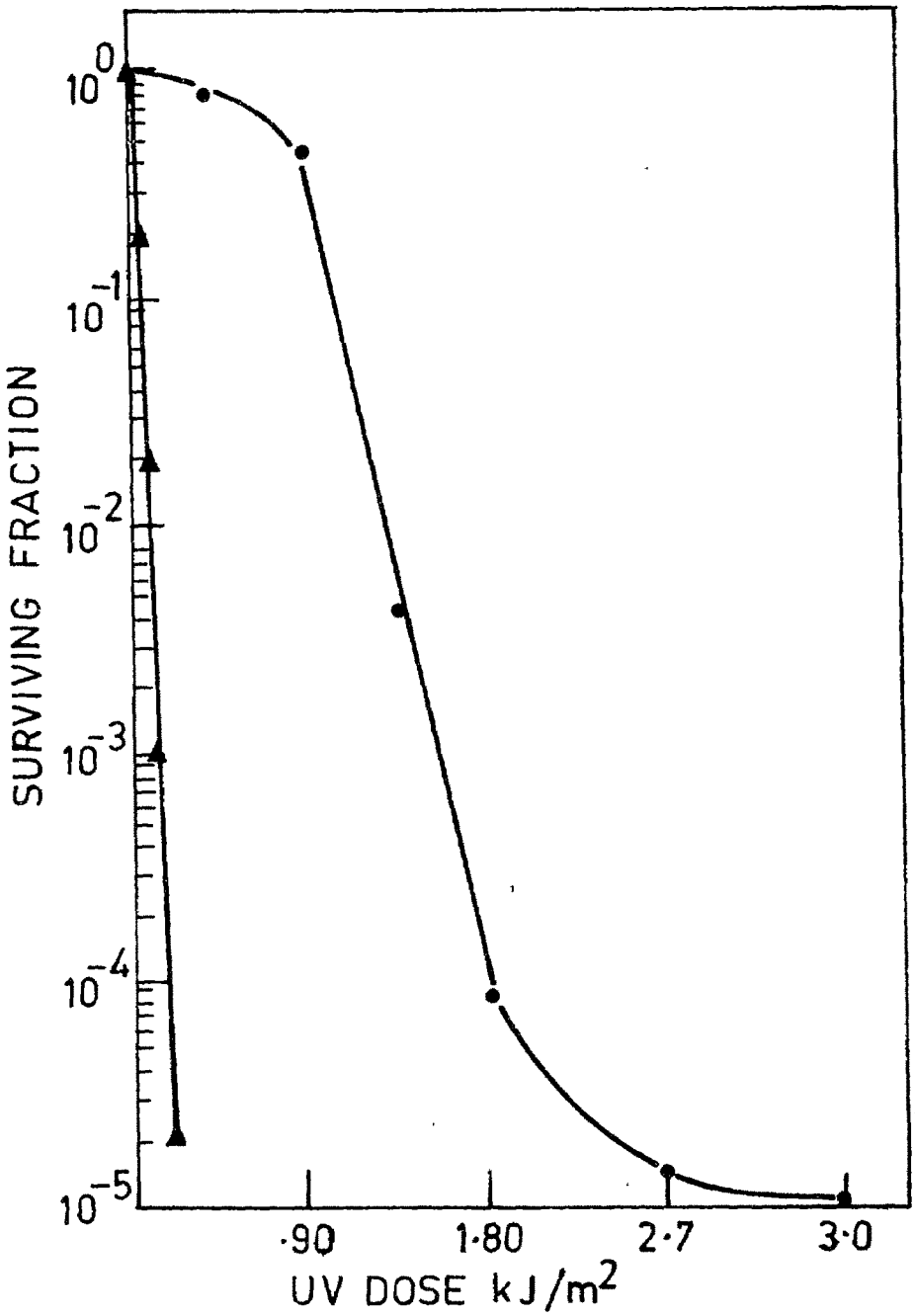


Figure 4. Survival of M.radiophilus and E.coli B/r exposed to UV radiation. Bacterial suspensions (7 ml) in 0.1 M phosphate buffer (pH 7.0) were exposed to various doses of UV radiations in glass petridish (dia. 9 cm) as described in the text. (-●-), M.radiophilus; (-▲-), E.coli B/r.

the UV resistance of this bacterium was comparable to that of M.radiodurans (results not shown). The broad shoulder is indicative of efficient DNA repair capability and it is quite likely that at least the limiting steps in the processes involved in the repairs of gamma radiation and UV radiation cellular DNA damages could be common. Apart from the broad shoulder region, another distinguishing characteristic of the UV survival curve was the presence of a long tail region appearing after 2500 $J/m^2$ and extending up to 5000 $J/m^2$ (the entire data are not given in the figure). It may be pointed out that such tail regions are absent in the case of UV radiation survival curves of moderately or highly radiation sensitive bacteria. Also such region was not found in the gamma radiation survival curve of M.radiophilus.

A variety of chemicals have been shown to be lethal to living cells mainly by their interactions with cellular DNA. In view of the possible existence of highly efficient DNA repair process in M.radiophilus, it was of interest to study the effect of a DNA-acting chemical on this organism. Comparative survival responses of M.radiophilus and E.coli B/r to various concentrations of an alkylating agent, methyl methanesulphonate, are depicted in Fig. 5. It is seen that at the MMS concentration of 50 mM, M.radiophilus is 20 times more resistant than E.coli B/r.

In view of the exceptionally high resistance that M.radiophilus had exhibited to gamma and UV radiations, as

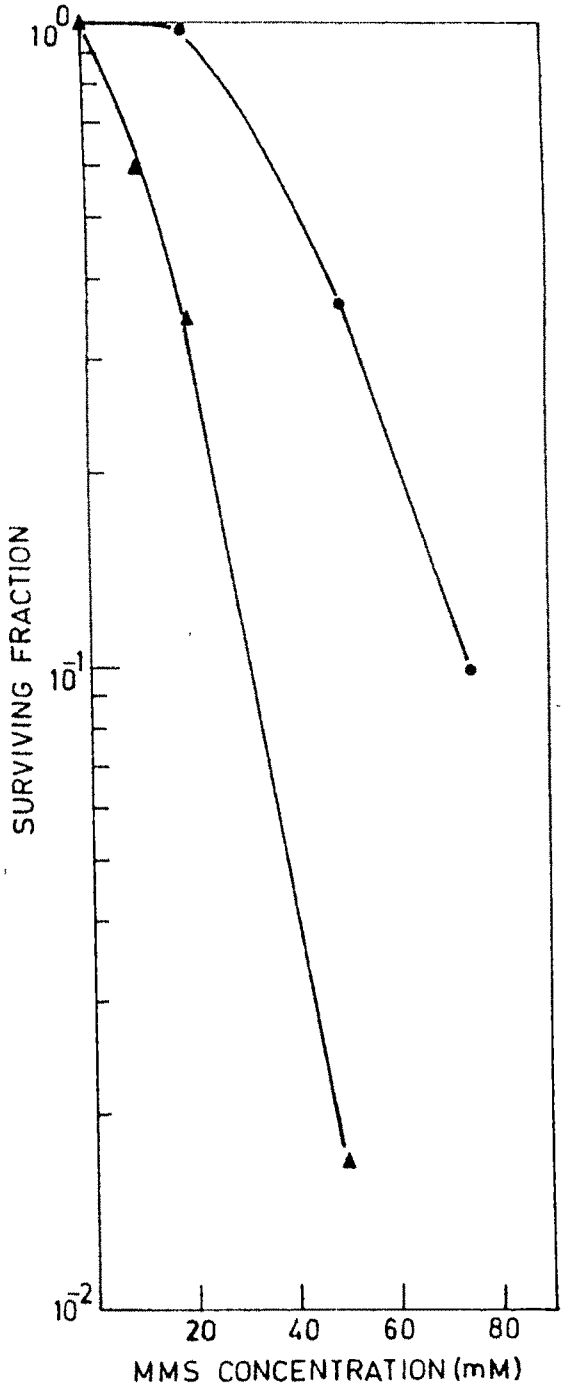


Figure 5. Survival of M.radiophilus and E.coli B/r treated with MMS. Bacterial cells from log phase culture were harvested, washed and incubated in TGYM medium containing various concentrations of MMS for 2 hours at 30°C. (-●-), M.radiophilus; (-▲-), E.coli B/r.

also to DNA-acting chemicals, it was thought of interest to examine if the organism could be resistant to other physico-chemical treatments. One such treatment that was studied in relation to the loss of viability of M.radiophilus cells was the subjection of the cells to repeated cycles of freezing and thawing. The results are illustrated in Fig. 6. It is clearly seen that as compared with E.coli B/r, M.radiophilus is much more resistant to freezing-thawing treatment. Whereas there was only a slight loss of colony-forming ability of M.radiophilus even after 25 cycles of freezing and thawing, E.coli cells population showed 3 log kill after only 4 such cycles. An explanation to such phenomenal resistance to a physical treatment could be ascribed to the greater thickness of the cell wall of M.radiophilus; electron microscopy has revealed a unique three layered cell wall structure of this organism.

As a further test to investigate the role of cell wall in the resistance of M.radiophilus to physical agents, the effect of ultrasonication of the viability of M.radiophilus cells was studied. The results are illustrated in Fig. 7. It is seen that cells (suspended in phosphate buffer at the density of $10^8$ cells/ml) were completely resistant to ultrasonication even to 20 kc/sec treatment for 20 min. The treatment of only one min ultrasonication sufficed to lyse E.coli cells suspended in phosphate buffer in similar manner.

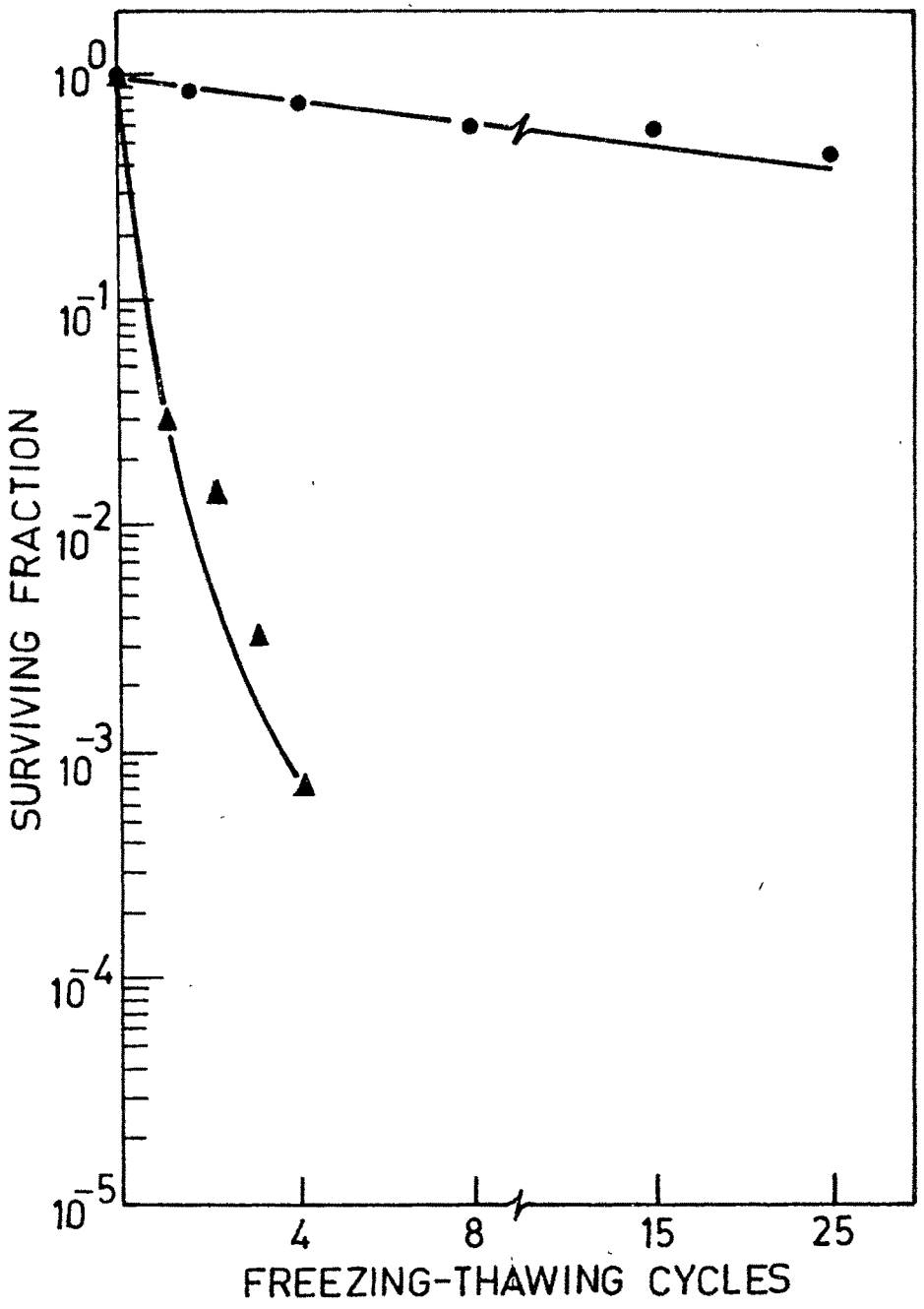


Figure 6. Survival of M.radiophilus and E.coli B/r subjected to various freezing and thawing cycles. Bacterial cells were suspended in phosphate buffer and frozen in liquid nitrogen. The frozen suspensions were thawed at 30°C. Survivals were determined after repeating the freezing-thawing cycles for different number of times. (-●-), M.radiophilus; (-▲-), E.coli B/r.

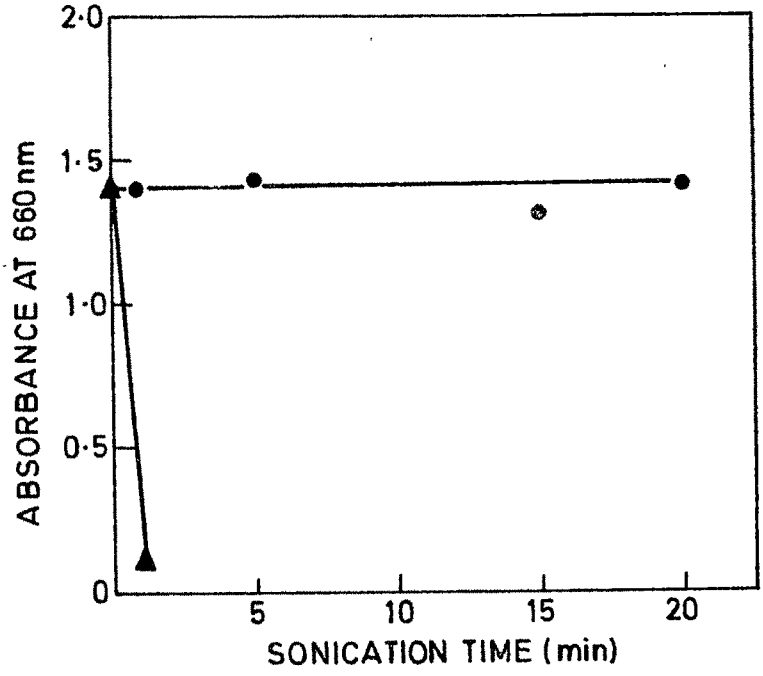


Figure 7. Survival of M.radiophilus and E.coli B/r subjected to ultrasonication. Bacterial cells were suspended in phosphate buffer at the density of $10^8$ cells/ml and survival was determined after ultrasonication for different lengths of time. (-●-), M.radiophilus; (-▲-), E.coli B/r.

The susceptibility of M.radiophilus to heating was also investigated. As seen in Fig. 8, there is more than 6 log reduction in the survival when M.radiophilus cells were subjected to heating at 60°C for 20 min. Thus this bacterium does not show particularly high resistance compared to other bacteria.

## DISCUSSION

Micrococcus radiophilus undoubtedly shows an exceptionally high resistance to gamma radiation comparable only with that shown by Micrococcus radiodurans. The $D_{10}$ value around 500 krad indicates that the organism is at least 15 to 20 times more resistant than E.coli B/r, the most resistant so far known among E.coli strains. As mentioned in the introduction to this chapter, the cell surfaces of radiation resistant bacteria are unique with a three-layered rigid cell wall structure (5, 6).

The rigidity of the cell wall structure - as evident from electron microscopy (5) was highlighted in the experiments involving freezing-thawing and ultrasonication. Compared to E.coli cells, M.radiophilus cells are quite refractory to these treatments mainly meant for rupturing the cell envelope. It is tempting to suggest that the rigid cell wall structure could afford protection to vital cellular targets, presumably DNA, against gamma irradiation attack.

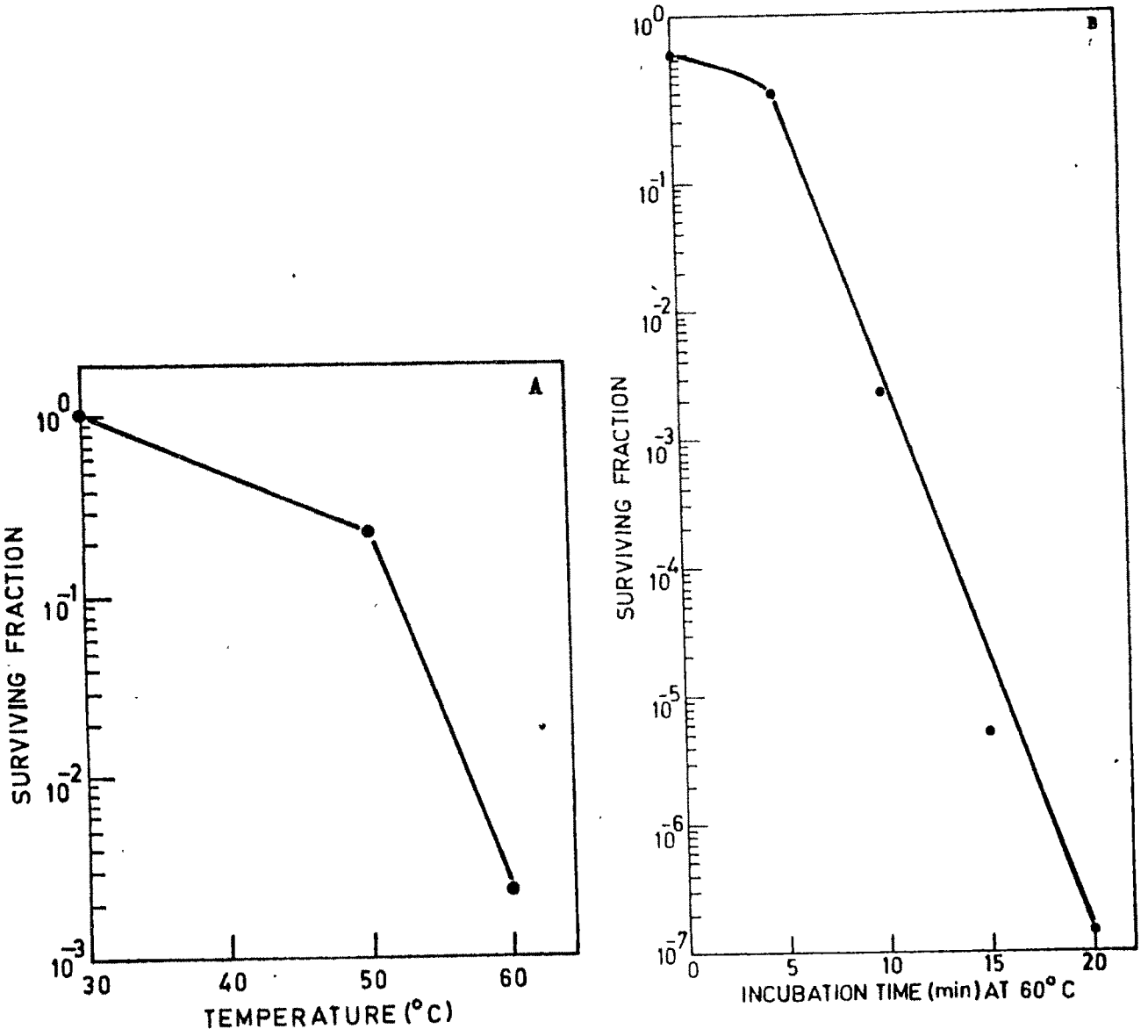


Figure 8. Heat sensitivity of M.radiophilus. Cells were suspended in 0.1 M phosphate buffer, pH 7.0, and their survival was determined. After incubation at (A) different temperatures for 10 min and (B) at 60°C for various times.

The organism also offers substantial resistance to ultraviolet radiation and MMS treatment when compared with the effects of these treatments on E.coli cells. Like gamma radiation, these treatments are known to cause cell death by injuring cellular DNA. It is hence pertinent to ask whether the rigid cell wall structure can serve as a protective shield to DNA against the damaging effects of all kinds of physical and chemical agents. Such a possibility however seems unlikely in view of the diverse types of mechanisms by which different physical and chemical agents seem to gain access across the cell wall and to bring about damage in the cellular DNA. The access of gamma rays to cellular DNA can be greatly impeded if the thick cell wall structure contained large amounts of sulphydryl-containing moieties. Ultraviolet radiation entry can be stopped if the cell wall had constituents that efficiently absorb UV radiation energy (for example, the UV radiation resistance of spores of certain bacteria is partly due to the presence of dipicolinic acid which absorb in the UV radiation region (16). The resistance of M.radiophilus cells to MMS observed in the present study could simply be interpreted as the result of a permeability barrier to this chemical. A number of studies with M.radiodurans suggest that this bacterium is highly resistant to almost all the DNA-acting chemicals so far examined (14, 17, 18). It is quite unlikely that the radiation resistance of M.radiodurans could be due to impermeability to all such chemicals. Similar arguments

may also hold good for M.radiophilus. At higher concentrations of MMS (still quite low from the permeability point of view), cells are susceptible to the chemical which implies that M.radiophilus may not be impermeable to MMS.

Taken together the results cast doubt on the theory that the cell wall structure may protect the DNA from the onslaughts by physical and chemical agents. Further work on the nature and composition of cell wall structure of radiation sensitive mutants of M.radiophilus and of M.radiodurans (the isolation of some has already been reported) (19) in comparison with similar studies on the parent radiation resistant strains may throw light on the role, if any, of cell surface structures in the determination of resistance to DNA-acting physical and chemical agents.

These considerations lead to the possibility of the existence of an efficient DNA repair machinery in M.radiophilus. Such DNA repair system(s) should be equally efficient in removing diverse types of lesions formed in cellular DNA in view of the finding that M.radiophilus, like M.radiodurans, is highly resistant to all kinds of DNA-damaging treatments.

The experiments on gamma radiation exposure given in split doses have given interesting insights into the recovery mechanisms in this organism. Whereas a single dose of 600 krad results in around 1% survival and of 900 krad in approximately 0.01% survival, the two doses of

600 krad and 300 krad with an intervening period of incubation in TGYM medium resulted in survival of 1%. Similar results were obtained when the order was reversed as follows: 300 krad - 2 hr in TGYM medium - 600 krad. The effect of 300 krad (which falls in the shoulder region) seems to be completely obliterated. Other experiments indicate that though a continuous radiation exposure of 700 krad results in 0.1% survival, the fractionated dose regimen of 400 krad - 2 hrs in growth medium - 300 krad resulted in 100% survival. Thus radiation exposure given in split doses is far less effective for lethal damage than when the same radiation exposure is given as a single dose. These results could be interpreted as the manifestation of DNA repair machinery. Yet they do not preclude the possibility that radiation resistance could be due to the mechanism by which cellular DNA is protected by damaging agents. However, another result of a split dose experiment supports the possibility of operation of DNA repair. The fractionated dose regimen of 400 krad - 2 hr incubation in buffer - 300 krad resulted in only 35% survival compared to 100% survival when the intervening period involved 2 hr incubation in TGYM medium. Thus a certain degree of DNA repair does occur during the period intervening the split doses, a component of DNA repair may depend on the growth medium and another could proceed even in a suitable buffer.

In E.coli, it has been shown that the inclusion of EDTA during the course of gamma irradiation of cells may

inhibit fast DNA repair - which can repair DNA strand-breaks in about 2 min in the absence of growth medium at 37° when compared with slow repair which may take about 40 - 60 min (15). In the present study, it was found that irradiation of cells along with EDTA resulted in small reduction of the shoulder region of survival curve thereby implying that fast repair may be a component of DNA repair armamentum of this organism. Since EDTA cannot be included in the plating medium used for the determination of viable count (this metal ion-chelating agent is bacteriostatic), [redacted] the sensitisation of cells to radiation could have been manifested only to a small extent. The aspects relating to DNA repair have been dealt with in greater detail in Chapter IV.

Coming back to the experiments on ultrasonication and freezing-thawing, it may be a fruitful exercise to ascertain whether there could be any tangible relationship between the resistance of M.radiophilus to the above treatments, on the one hand, and to its resistance to various DNA-damaging agents, on the other. In other words, is it possible that the exceptionally high resistance that this organism displays to ultrasonication and freezing-thawing could arise from the powerful DNA repair machinery that it seems to possess ? There have been sporadic reports which indicate that freezing-thawing may cause loss of cell viability due to the production of strand breaks in DNA (20, 21).

The resistance offered by M.radiophilus to freezing-thawing could be due to the possibility that strand breaks created by this treatment could be efficiently repaired. Similar mechanism can be envisaged in respect of the high resistance that M.radiophilus exhibits to ultrasonication.

Although it is generally believed that heat-inactivation of bacterial cells could be due to protein denaturation, there have been reports claiming that strand breaks in DNA may be the primary lesion responsible for the loss of cell viability. In the present study, it was found that unlike its responses to freezing-thawing and ultra-sonication, the responses of M.radiophilus to heat did not reveal any exceptional heat resistance. Either the heat-induced DNA strand breaks may not be an important contri-butory factor in this microorganism, or that the DNA repair complex could be heat-sensitive. The behaviour shown by this organism to heat is in line with the fact that some strains of C.botulinum are as much more radiation-resistant than C.sporogenes as is C.sporogenes more heat-resistant than C.botulinum (22). The opposing behaviour to radiation and heat can be a general rule; if so this may have impli-cations in the development of suitable methods for food preservation based on combination of radiation and heat treatments.

Finally it would seem appropriate to discuss certain general aspects arising from the high radiation

resistance of M.radiophilus. At high non-lethal radiation dose (e.g. 400 krad), it is possible that some non-DNA components of the cell could have been damaged. The fact that there is no loss of cell viability could mean: (i) that proteins and other non-DNA components irrespective of whether they are from radiation sensitive or resistant microbes are much more resistant compared to cellular DNA, or (ii) that proteins and other non-DNA components from radiation resistant microorganisms may also be more resistant to radiation compared to their counterparts in radiation sensitive microorganisms. In this context, it is pertinent to discuss the properties of microorganisms known to grow in abnormal physical and chemical environments. Studies on the kinetics of thermal denaturation both of enzymes and of cell structures that contain proteins (e.g., flagella, ribosomes) have shown that many specific proteins of thermophilic bacteria are considerably more heat-stable than their homologues from mesophilic bacteria (23). Also interestingly, in certain sporulating bacteria, the same enzyme may be heat-sensitive in vegetative cells but heat-resistant in spores (24). In the extreme halophiles, which require high concentrations of $Na^+$ for growth, several enzymes (e.g., malic dehydrogenase) also seem to require high concentration of NaCl for optimum catalytic activity (23).

It is worthwhile examining whether in the phenomenally high radiation resistant microorganisms, at least

some of the cellular components, such as enzymes, can be also resistant to radiation-inactivation. Attempts have been made in studies to be described in the next chapter to seek answers to some of these questions.

## SUMMARY

Studies were conducted to ascertain the effects of gamma/UV radiations and other physical/chemical treatments on the survival responses of M.radiophilus, an organism isolated in this laboratory from Bombay duck (Harpodon nehereus). This organism showed exceptionally high radiation resistance to gamma radiation, being 15 to 20 times more resistant than E.coli B/r. The log survival curves ~~were~~ exhibited an unusually long shoulder region extending upto 400 krad and the $D_{10}$ value (10% survival) was 500 krad. Whereas a single dose of 700 krad resulted in the reduction of survival upto 0.1%, when the same dose was split into two fractions of 400 and 300 krad, with an intervening 2 hr incubation in TGYM medium the survival was about 95%. When, however same doses were given in with an intervening period in phosphate buffer (instead of TGYM broth) the survival was about 35%. Inclusion of EDTA an inhibitor of fast repair in E.coli during gamma irradiation resulted in small but significant sensitization of M.radiophilus cells. These results are indicative of efficient repair machinery. M.radiophilus cells were also found to be highly resistant to UV radiation (shoulder region extending up to 800 $J/m^2$ with the $D_{10}$ value of 1120 $J/m^2$). The organism showed high resistance to the treatment with methyl methane sulfonate an alkylating agent whose lethal effect is known to be due

to damage to DNA. The organism was highly resistant to the repeated freeze-thaw cycles and also to prolonged ultrasonication treatment. The resistance to these treatments could be attributed to the presence of rigid cell wall structure. This bacterium did not show particularly high temperature resistance compared to other bacteria.

REFERENCES


1. Anderson, A.W., Nordon, H.C., Cain, R.F., Parrish, G. and Duggan, D. (1956) Food Technology 10, 575.
2. Duggan, D.E., Anderson, A.W., Elliker, P.R. and Cain, R.F. (1959) Food Research 24, 376.
3. Lewis, N.F. (1971) J. Gen. Microbiol. 66, 29.
4. Setlow, J.K. and Duggan, D.E. (1964) Biochem. Biophys. Acta 87, 664.
5. Lewis, N.F., Shah, A.R. and Kumta, U.S. (1975) Ind. J. Microbiol. 15, 37.
6. Lewis, N.F., Alur, M.D. and Kumta, U.S. (1973) Can. J. Microbiol. 20, 455.
7. Bruce, A.K., Sansone, P.A. and Maevitle, T.J. (1969) Rad. Res. 38, 95.
8. Baird-Parker, A.C. and Holbrook (1971) In "Inhibition and destruction of microbial cell", ed. Hugo, W.B., Academic Press, p. 369.
9. Sleyter, U.B., Silva, M.T., Kocur, H. and Lewis, N.F. (1976) Arch. Microbiol. 107, 313.
10. Thornley, M.J., Horne, R.W. and Glauret, A.M. (1965) Arch. Fur Microbiologie 51, 267.
11. Lancy, P. Jr. and Murray, R.G.E. (1978) Can. J. Microbiol. 24, 162.
12. Thompson, B.G., Anderson, R. and Murray, R.G.F. (1980) Can. J. Microbiol. 26, 1408.
13. Ito, H. (1971) Agri. Biol. Chem. 35, 1566.

14. Tempest, P.R. and Moseley, B.E.B. (1980) Mol. Gen. Genet. 179, 191.

15. Town, C.D., Smith, K.C. and Kaplan, H.S. (1974) Curr. Topics in Rad. Res. Vol. VIII, 353.

16. Grecz, N., Tang,Tang, T. and Frank, H.A. (1973) J. Bacteriol. 113, 1058.

17. Sweet, D.M. and Moseley, B.E.B. (1974) Mut. Res. 23, 311.

18. Sweet, D.M. and Moseley, B.E.B. (1976) Mut. Res. 34, 175.

19. Moseley, B.E.B. (1967) J. Gen. Microbiol. 49, 203.

20. Alur, M.D. and Grecz, N. (1975) Biochem. Biophys. Res. Commun. 62, 308.

21. Alur, M.D., Lewis, N.F. and Grecz, N. (1977) FEMS Microbiol. Letters 1, 367.

22. Goldblith, S.A. (1971) In "Inhibition and destruction of the microbial cell", Academic Press, p.285.

23. Stanier, R.Y., Adelberg, E.A., Ingraham, J.L. (1977) In "General Microbiology", Macmillan Press, Pg.293.

24. Sadolf, H.L. (1970) J. Appl. Bacteriol. 33, 130.