CONCLUDING REMARKS

The genotoxic potential of 4 species of bacteria studied in 2 species of fish, *Oreochromis mossambicus* and *Anabas testudineus* in more detail and in 3 species of Indian major carps viz. *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* by MNT only, has reasonably been revealed. Almost in all the tests, positive results were encountered which have been discussed already while presenting the data. Since living mutagens as a new category of mutagen added to physical and chemical agents, they deserve some considerations in including them as mutagenic agent in the field of mutagenesis. The point mutation induced by living agent has not yet been evidenced so clearly like that induced by physical and chemical agents but the chromosomal mutations induced by living mutagens are beyond question, which have been reviewed in mammalian models by different workers (Aula, 1965; Bartsch, 1970; Nichols, 1966, 1968, 1978; Manna, 1973, 1980, 1982, 1989a, 1992a). Just to point out the similarities in chromosomal mutation induced by chemicals and the living mutagens like bacteria and fungi in mice system, the following common features of the effect could be summarized: i) Breaks were overwhelmingly chromatid types. ii) MNT was positive and the effect was somewhat dose-dependent. iii) The individual type aberrations were non-randomly distributed between or within chromosomes. iv) The lethal mutations could be detected in F₁ and F₂ generations. v) The living individuals of F₁ and F₂ generations showed the similar genetic effect. vi) The genic involvement in such manifestations has been discussed (Manna, 1981, 1984, 1986a, 1989a).

The present study also showed the similarity in the genotoxic effect induced by different species of bacteria with that of the chemicals reported earlier (Manna and Sadhukhan, 1986a, b, c; Sadhukhan and Manna, 1986, 1989; Manna and Mukherjee, 1986, 1989a, b). The main similarities found in the cytogenetic assays induced by chemical, bacterial and fungal agents assessed mainly in *O. mossambicus* as a model (Manna, 1986b) were as follows: i) Breaks were mainly chromatid type, and somewhat nonrandomly distributed. ii) Meiotic chromosome aberrations were positive. iii) MNT was
iv) Dominant lethal test was positive. Therefore, living mutagen in aquatic environment on fish has more or less reasonably been established by the present study while mutagenic effects of chemicals have been reported by various workers in fish (Manna and his collaborators, lit cit; Kligerman, 1979, 1982; Hooftman and de Raat, 1982; Kocan et al., 1982; Longwell, 1977; Manna, 1989b; Prein et al., 1978).

Though the mutagenic potential of chemical and living mutagens like bacteria and fungi has been recorded as mentioned above, the precise mechanism of action remains unresolved. The genotoxic effects of the chemical mutagens have been discussed to be induced by means of biochemical action mediated through DNA (Kihlman, 1966). Since at present, thousands of chemicals and drugs of odd constitution and diversified nature have been found to induce genotoxic effects more or less of similar nature, the idea of the genotoxic effect of different chemicals mediated through biochemical actions of specific nature, seemed no longer tenable because many chemicals have no effect on nucleic acid. Therefore, some common mode of action has been envisaged (Manna, 1975). In the same sense, microbes belonging to different groups like viruses, bacteria, fungi etc showed the similarity in genotoxic effects compared with those of chemical agents, for which some common mode of action could be considered to explain the genotoxic effect induced by chemical and living mutagens. However, we could not suggest any working hypothesis for the commonness in effect induced by chemical and living mutagens other than the effect of stress as suggested by Manna (1975). Anyhow, we focussed the problem for receiving future attention in the light of our expanded knowledge of mutagenesis.

Among microbes, viruses being very small have access to host nuclear chromosome, while bacteria and eukaryotic microorganisms would be too large to have direct access into the nucleus and affect the host nuclear chromosomes. Therefore, it seemed that odd kinds of microbes might be producing some physico-chemical stress which could have affected the chromosome structure. It can also be thought of that if the stress effect could induce mutational change in some chromosome loci of the host cell, that change might result in producing various types of cytogenetic manifestations. If it is envisaged that the maintenance of structural integrity
of chromosome is under genic control and if the gene concerned undergoes mutational change by odd living and chemical agents, various types of chromosome aberrations might be resulted because there would be no control in maintaining structural integrity of chromosome for the mutational change of the gene concerned. The gene concerned might also have pleiotropic effect because the effects were found in chromosome aberrations, sperm head morphology etc. The occurrence of the similar nature of cytogenetic effects found in $F_1$ and $F_2$ generations of mice whose male parents were treated with some chemicals, bacteria and fungi by Manna and his collaborators (Dey, 1981; Roy, 1983; Banerjee, 1983; Ghosh, 1985; Chatterjee, 1987; Sarkar, 1988; Kundu, 1988; Panda, 1989; Banerjee, 1989; Pal, 1990) in mice has been elaborately discussed and some hypothesis has been advocated to explain them by Manna (1984, 1986a, 1989a).

As found with chemical mutagen the genotoxic potential differed greatly and with this in view, we compared the relative sensitivity by analyzing the results of the cytogenetic data induced by 4 different species of bacteria in fish.

In *Oreochromis mossambicus* : We have already compared the relative genotoxic effects of 4 species of bacteria after treatment to *O. mossambicus*. Here we have compared data of some common interval with a view to make the statistical test. The chromosome aberration frequencies at 24 hr in gill epithelial cells of *O. mossambicus* treated with 4 different species of bacteria were compared (Table 72A) and it was found that *P. aeruginosa* (17.5%) had the maximum effect followed by *B. subtilis* (14%), *S. aureus* (13%) and *B. acida* (11%). A comparative statistical analysis of the aberration data for *P. aeruginosa* vs *B. subtilis*, *P. aeruginosa* vs *S. aureus*, *B. subtilis* vs *S. aureus*, *B. subtilis* vs *B. acida* and *S. aureus* vs *B. acida* revealed that the difference was statistically not significant ($P > 0.05$), when only the difference in the data between *P. aeruginosa* and *B. acida* was significant ($P < 0.05$) indicating thereby that the genotoxic potential of *P. aeruginosa* was strongest and that of *B. acida* was weakest among four species of bacteria tested. However, the frequency data of net increase of chromosome aberration (Table 72A) which have already been compared, was 9.0% for the treatment of *P. aeruginosa*, 5.5% for *B. subtilis*, 7.0% for *S. aureus* and 5.0% for *B.
Table 72 Compiled data of cytogenetical studies at 24 hr on A. *Oreochromis mossambicus* and B. *Anabas testudineus* treated with 4 species of bacteria, and lethal effect on eggs and developing embryos leading to unfertilized and dead ones respectively in the combined data of 4 week mating.

A. *Oreochromis mossambicus*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Chr. aberr. in gill</th>
<th>Chr. aberr. in Met-I</th>
<th>MNT(%)</th>
<th>L.E(%)</th>
<th>Eggs</th>
<th>Developing embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trea (%)</td>
<td>Net incr (%)</td>
<td>Trea (%)</td>
<td>Net incr (%)</td>
<td>Bl</td>
<td>G</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>17.5</td>
<td>9.0</td>
<td>28.5</td>
<td>11.0</td>
<td>0.31</td>
<td>0.33</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>14.0</td>
<td>5.5</td>
<td>28.0</td>
<td>10.0</td>
<td>0.16</td>
<td>0.1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>13.0</td>
<td>7.0</td>
<td>25.0</td>
<td>7.5</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td><em>B. acida</em></td>
<td>11.0</td>
<td>5.0</td>
<td>24.5</td>
<td>11.0</td>
<td>0.06</td>
<td>0.08</td>
</tr>
</tbody>
</table>

B. *Anabas testudineus*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Chr. aberr. in kidney</th>
<th>MNT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trea (%)</td>
<td>Net incr (%)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>17.5</td>
<td>12.2</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>11.3</td>
<td>7.3</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>10.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>B. acida</em></td>
<td>8.3</td>
<td>4.0</td>
</tr>
</tbody>
</table>
acida, showing no significant difference between any two net increase data.

Similarly, a comparison of the 1st spermatocyte metaphase chromosome aberration frequency induced for the treatment of 4 species of bacteria (Table 72A) was found statistically not significant (P > 0.05) which agreed with the comparative data of somatic chromosome aberration except that of P. aeruginosa vs B. acida. Further, if the frequency of net increase in treated series over control was considered it was 11.0% for P. aeruginosa, 10.0% for B. subtilis 7.5% for S. aureus and 11.0% for B. acida treatment which showed that the net increase frequencies induced by 3 species of bacteria were very close except that of S. aureus (Table 72A) but the differences between the data of net increase as well as of treated series for any two bacteria were statistically not significant.

The frequency of micronucleated erythrocytes induced by the treatment of 4 species of bacteria at 24 hr was 0.31%, 0.16%, 0.13% and 0.06% respectively (Table 72). A comparative study of the data revealed different levels of significance as it was less significant (P < 0.05) for P. aeruginosa vs B. subtilis and also for P. aeruginosa vs S. aureus, and highly significant (P < 0.001) for P. aeruginosa vs B. acida while the difference was statistically not significant for other comparison. Similarly, the MNT data in gill epithelia was 0.33%, 0.1%, 0.13% and 0.08% for the treatment of 4 species of bacteria respectively (Table 72A). A comparison of the data showed that the difference was significant at different levels as it was significant (P < 0.005) for P. aeruginosa vs B. subtilis, less significant (P < 0.05) for P. aeruginosa vs S. aureus and highly significant (P < 0.001) for P. aeruginosa vs B. acida, while the difference was not significant for others. Therefore, the comparison of MNT data of gill epithelia showed the same trend like that of blood. The MNT data of kidney cells for the treatment of 4 species of bacteria yielded 0.13%, 0.05%, 0.06% and 0.03% respectively. A comparison of the data revealed that only the difference between P. aeruginosa vs B. acida was significant (P < 0.05) while in all other cases the difference was not statistically significant.

Besides the data of cytogenetic assays like somatic chromosome aberration, meiotic chromosome aberration and MNT, the data of lethal test
also showed some difference because in the combined data of 80 females, the lethal effect on eggs leading to unfertilized ones was 0.518% for treatment of *P. aeruginosa*, 0.511% for *B. subtilis*, 0.48% for *S. aureus* and 0.27% for *B. acida* while the lethal effect on developing embryos leading to dead ones was 0.37%, 0.32%, 0.26% and 0.09% respectively. The differences in the data of unfertilized eggs as well as dead embryos were very striking between *P. aeruginosa* and *B. acida* which clearly indicated, as pointed out before, that the genotoxic potential of *P. aeruginosa* was strongest and that of *B. acida* was weakest.

Therefore, the compiled data of cytogenetic assays (Table 72A) in *O. mossambicus* showed maximum effect for the treatment of *P. aeruginosa* as found in all the testing protocols and minimum for *B. acida*, which was also, in many cases, supported by statistical test. On the other hand, the data of cytogenetic assays for the treatment of *B. subtilis* was lower than that for the treatment of *P. aeruginosa* but the difference was not steadily maintained and in some tests the difference was not at all striking. Similarly, for *S. aureus*, the data of cytogenetic assays was little lower than that for *B. subtilis* in most of the testing protocols except in metaphase I and MNT of gill epithelia. Thus, the data of cytogenetic assays revealed, on the whole that *P. aeruginosa* had the strongest genotoxic potential and *B. acida* had the minimum while *S. aureus* and *B. subtilis* showed the genotoxic potential in between the two extremes i.e. *P. aeruginosa* and *B. acida* in *O. mossambicus* used as testing model.

In *Anabas testudineus*: The chromosome aberration frequency in kidney cells of *A. testudineus* treated with 4 species of bacteria, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *B. acida* was also compared and it was found that, like *O. mossambicus* more or less the same trend was maintained (Table 72A and B) in showing the maximum effect for the treatment of *P. aeruginosa* and the minimum for the treatment of *B. acida*. The net increase in aberration frequency was 12.2%, 7.3%, 6.0% and 4.0% respectively while the frequency in treated specimens was 17.5%, 11.3%, 10.0% and 8.3% respectively. A comparison of somatic chromosome aberration data showed that the difference in the frequency was less significant (P<0.05) for *P. aeruginosa* vs *B. subtilis* significant (P<0.005) for *P. aeruginosa* vs *S. aureus* and highly significant
(P < 0.001) for *P. aeruginosa* vs *B. acida* indicating that *P. aeruginosa* was strongest and *B. acida* was weakest genotoxic agents among 4 species tested. The difference in aberration frequency was statistically not significant for *B. subtilis* vs *S. aureus*, *B. subtilis* vs *B. acida* and *S. aureus* vs *B. acida*.

The MNT in peripheral erythrocytes, however, showed no significant difference between the data of any two treated series except that of *P. aeruginosa* vs *B. acida* and *B. subtilis* vs *B. acida* (Table 72B). In the gill epithelia, there was no significant difference for any 2 treated series, while in kidney, except the difference for *P. aeruginosa* vs *S. aureus* (P < 0.05), others were not significant.

Thus the statistical analysis of the data of different cytogenetical tests between 2 species of bacteria did not show uniform result except for *B. subtilis* vs *S. aureus*, *B. subtilis* vs *B. acida* and *S. aureus* vs *B. acida* where almost no significant difference was encountered. There were several factors which might have influenced the data to some extent.

When the data of genotoxic potential of a particular species of bacteria in 2 species of fish, *A. testudineus* and *O. mossambicus* for the somatic chromosome aberration frequency and MNT at 24 hr (Table 72A,B) were compared, it was found that, very broadly, *O. mossambicus* was little more vulnerable than *Anabas* but the difference was not significant except for the data of MNT of peripheral erythrocytes (P < 0.05) and gill (P < 0.001) for the treatment of *P. aeruginosa* and the data of MNT of kidney (P < 0.05) for *S. aureus* treatment. Therefore, in spite of some significant difference in few data, in majority of the cases the difference was not significant. Therefore, it appears that no significant difference could be established for the sensitivity of *O. mossambicus* and *A. testudineus* to the treatment of a particular species of bacteria.

Therefore, on the whole, it could be concluded from the comparison of the data of cytogenetical assays that the different species of bacteria viz. *P. aeruginosa*, *B. subtilis*, *S. aureus* and *B. acida*, had somewhat different degree of genotoxic potential, of which the strongest one was *P. aeruginosa* shown by every test and weakest one was *B. acida*. On the other hand, species sensitivity of fish i.e. between *O. mossambicus* and *A.
testudineus, was not found to be very distinct one.

Non-random distribution of individual type chromosome aberrations in tilapia: While scoring the somatic chromosome aberration data of tilapia it appeared that some individual type aberrations comprising break, constriction and gap were nonrandomly distributed for which some critical analyses of the above individual type aberrations in 2 marker and 42 non-marker chromosomes induced by 4 species of bacteria were made. In a total of 1600 metaphase plates examined in gill epithelial cells of O. mossambicus induced by P. aeruginosa, B. subtilis, S. aureus and B. acid, 25 individual type aberrations were encountered in marker chromosomes and 21 in nonmarker group. Thus, in the total of 46 break, gap and constriction type individual aberrations, if the distribution was random, the expected number would have been 2 in marker chromosomes and approximately 44 in non-marker chromosomes, while the number observed was 25 in marker and 21 in nonmarker chromosomes. Therefore, the difference between the observed and expected numbers was very glaring which clearly indicated that marker chromosomes were more vulnerable than the non-marker group. The size of 2 marker chromosomes was relatively very large which was calculated to be 1/10th of total genome length (Manna and Som, 1982). Therefore, according to genome length the number of the expected individual type aberrations should have been approximately 5 in marker but the observed number was 25 which was 5 times higher than that of the expected value. In non-marker chromosomes, according to genome length the expected number of individual type aberrations should have been about 40 against which only 21 or about half of the expected number were observed. Therefore, the data clearly indicated that the individual type aberrations induced by different species of bacteria were non-randomly distributed. As the frequency was very low, the data had to be pooled. Similar trend of non-random distribution of individual type aberrations in chromosomes of tilapia induced by x-rays was also recorded (Manna and Som, 1982).

The regionwise distribution of the chromatid breaks, constrictions, and gaps in the marker chromosome was analyzed with a view to finding out if it was also non-random. So, arbitrarily, the marker chromosome was divided lengthwise into 3 equal regions as proximal including the
centromere, middle and the distal ones. Out of the total of 25 individual type aberrations encountered in the marker chromosomes, 17 were in the distal region and 8 in the middle region, while there was none in the proximal region. If such aberrations were random in occurrence, each region should have the same number of about 8, but the data revealed that the distal region had 2 times more than the expected number of 8, the midregion had the same as expected number, and the proximal region had none or in other words not the least vulnerable. Thus, we could, very broadly, generalize that the distal region was most vulnerable to the genotoxic effect of different bacteria. Manna and Som (1982) reported the similar type of regionwise non-random distribution of individual type chromosome aberrations in gill epithelial cells of X-irradiated tilapia. The present data and the X-ray data showed the similar trends that the marker chromosomes were relatively more susceptible than non-markers and their distal region had the maximum vulnerability to the effect of mutagens.

Similar analysis of groupwise and regionwise distribution of chromatid breaks in mice induced by good number of chemical and living mutagens, led Manna (1981) to claim the presence of 'Inherent Weaker Region' in mouse karyotype. The analysis of individual type chromosome aberrations in tilapia also supported the same hypothesis. In recent years, some 'Hot Spot' or 'Weaker-Region' have been claimed as fragile sites specially in human karyotype, which have been reviewed by Manna (1989c). The cases of regular occurrence of breaks in chromosomes as found in animal species like ascaris, cyclops and mammals including man have been claimed by different authors (White, 1973; Manna, 1989c; Ahuja et al., 1989; Yunis, 1985). The occurrence and importance of fragile sites in chromosomes of man have been reviewed by some workers (Sutherland and Hecht, 1985; Yunis, 1985, 1988; Ahuja, 1988; Ahuja and Prasad, 1991). More than 138 fragile sites have been identified in human genome (Ahuja et al., 1989). They have been probed for congenital malformation and site of oncogene in cancer. The inherent weaker region in the structure of prokaryotic and eukaryotic chromosomes has been reviewed by Manna (1989c). It has been found in Lambda phage, Drosophila, some invertebrates, mammals specially in mouse and man, and also in higher apes while it has also been envisaged
in the fish tilapia when chromosome breaks were analyzed after their induction by X-rays and the living mutagens.

The genotoxic potential of 4 species of bacteria in 5 species of fish viz. *O. mossambicus*, *A. testudineus*, *L. rohita*, *C. catla* and *C. mrigala* might have some other implications. It is yet to be seen if infection of the bacteria might induce some chromosome aberrations in the infected person because there is evidence that the culture of 2 bacterial species under genera *Pseudomonas* and *Micrococcus*, isolated from tumour of *A. testudineus* induced chromosome aberrations in mice when they were treated separately (Manna and Pal, 1982). The chromosome aberration in the fish with tumour was also recorded. This would lead to expect that some of these bacteria would also show genotoxic effect in fish infected with them, as such genotoxic effects have been shown by some virus in the leucocyte culture of infected person as well as by the bacteria in the cultured cells of mammals infected with them (Bartsch, 1970; Manna, 1973). Further, tubercle bacilli, *Mycobacterium tuberculosis* was found to be potential genotoxic agent by a battery of tests in the experimentally treated mice (Manna and Pal, 1989, 1990a,b, 1991); the chromosome aberrations were also reported in cultured cells of patients suffering from tuberculosis (Jaju et al., 1984). Recently, it has been found in our laboratory that a nitrogen fixing bacterium, *Xanthobacter autotrophicus* which was found to be genotoxic to mouse by a battery of tests (Sadhukhan and Manna, 1986; Manna and Sadhukhan, 1989, 1991) induced good amount of chromosome aberrations in human leucocyte culture infected with the bacteria (Manna and Sadhukhan, unpublished).

Therefore, though the present study was conducted on experimental fish after treatment with bacteria, their natural infection to fish might also cause genotoxic effect. Sometimes, fish mortalities due to different types of microbial infection were reported (Roberts, 1978). So, it would be worthwhile to see if such infected fish carried also some genotoxic effect or not. Thus the implication of the study to mankind is yet to be established though it has been reported that Japanese people consuming fish exposed to methyl mercuric poison, showed some chromosome aberrations in their leucocyte culture (Skerving et al., 1970).
Lastly, it may be argued that in the present study, the cultures of different spp. of bacteria were intraperitoneally injected in fish though the normal site of infection of 3 species of bacteria, *P. aeruginosa*, *S. aureus* and *B. subtilis* (their pathogenicity and etiology very rarely reported) might not be the peritoneal. Yet, we have introduced the bacteria intraperitoneally because the effect was shown quickly and it has been the general way of treatment of chemicals to assay their genotoxic potentials. It is also known that many bacteria even some viruses, fungi living in harmony within human body form the microbial ecosystem there, which is studied under the discipline, 'Gnotobiology'. There are more than 15 different species of bacteria belonging to pneumococci and streptococci groups, and viruses of adenovirus group normally live in our mouth and upper respiratory tract, but they cause serious diseases when deranged from their normal site in man. Even the non-pathogenic *Escherichia coli* which is beneficial to our body, on derangement causes various diseases and it has also been found to be genotoxic (Manna, 1992b). Therefore, the effect that was found by introducing the bacteria to unnatural site, was just to show the genotoxic potential of the bacteria. The present study of genotoxic potential of bacteria in experimental fish would at least draw the attention to this utterly neglected field, the importance and implication of which need no emphasis.