THE NEW HORIZON OF MUTAGENESIS

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MUTAGENS are older than the life itself and mutation stands at the root in the evolution of life processes. It appears from our present knowledge that no environment is free from one form of mutagen or the other. The situation has become more alarming for the ever increasing atomic and chemical practices relating to agriculture, public health and industries to which the role of 'Living Mutagens'[1,2] concerning our present theme has to be added as this has not received due attention uptil now. A Dutch botanist, Hugo De-Vries, at the beginning of the 20th century discovered some sudden heritable unusual change in otherwise fairly uniform natural population of the plant, *Oenothera lamarckiana* and this change was considered to be due to mutation and thus the mutation theory was proposed. However, the case of *Oenothera* was later found to have arisen from chromosomal abnormalities and not from true gene mutation.

The artificial manipulation of mutation began after Muller's[3] fundamental discovery of the x-ray induced mutation in *Drosophila* and a great deal of progress has also been made on the radiation induced chromosome aberrations pertaining to the mechanism of action, their behaviour during mitotic and meiotic cell division, the fate in subsequent cell generation, the problem of radioprotection etc. Similarly the discovery of the induction of polyploidy for the treatment of colchicine[4], chromosome aberrations by urethane[5] and the gene mutation by nitrogen mustard[6] opened the second front of mutagenesis by chemical agents. This had a greater impact because the number of chemical mutagens in human environment has been too large as compared to the other physical agents. Traditionally the mutagens are classified into the physical and the chemical agents; but the pioneering discoveries of the induction of chromosome aberrations (a) in cultured cells of hamster, infected with Herpes simplex virus[7], (b) in spermatocytes of insects infected with ricketssia[8] and mycoplasma[9], and (c) in bone marrow cells of mice, injected with the culture of the human pathogenic bacterium, *Staphylococcus aureus*[2,10,11] and spores of the fungus *Aspergillus fumigatus*[11] paved the way, for what has been focussed here as 'The new horizon of mutagenesis' relating to these 'Living mutagens'[1,2,12].

Until about the last decade, the mutagenic effects of the physical, chemical and living agents have traditionally been assayed only by the chromosome abberrations because this method was standardised long before, dependable, economical, easier in handling and for its direct involvement in the genetic system; but some workers remarked the method as tedious, time consuming, etc. The chromosomal damage involving structural change designated as clastogenic effect, implied a direct effect in the genetic apparatus while those agents causing the numerical change as 'mitotic poisons'; but some agents might bring both types of changes. Therefore, the effect was not very mutagen specific. Similarly the grouping of chemicals as mutagenic, carcinogenic and teratogenic is also artificial as the same agent might induce all the three effects. Anyhow, the alarming degree of environmental pollution by the use of hundreds of chemicals and by the radiation energy in agriculture, public health, personal habits of drugs and cosmetics, industries, etc., has created a new field of genetics—'The genetic toxicology'. In terms of public health the genetic toxicology is now being investigated for (a) mutagens in our environment, (b) mutagenicity of pesticides, (c) mutagenicity of drugs, (d) mutagenicity of cosmetics, (e) mutagenicity of food stuff and food preservatives, (f) mutagenicity of industrial effluents and exhausts, (g) mutagenicity of sewerage disposal. Therefore, various testing methods for mutagenicity have been developed, little over a decade, to cope up with the demand for the

public safety and health and to avoid the hereditary hazards [13-17]. Among the different mutagenicity testing methods developed, other than the classical chromosome aberration testing one, the commonly used ones are as follows:- (i) *The determination of the host-mediated assay* in which the ability of the laboratory animals to potentiate or detoxify a compound with regard to their mutagenic activity. There are compounds which might not be mutagenic in microorganisms but are converted to active mutagen in animals or *vice-versa*. The testing chemicals and the microorganisms are administered by different routes; and after a period the mutation if induced is determined on withdrawing the microorganisms from the mammal. (ii) *The DNA repair assay*—as a correlation exists between the mutagenicity of an agent and its capacity to induce non-semiconservative DNA synthesis in cultured mammalian cells, the monitoring of the unscheduled DNA synthesis provides a means for identifying the mutagenicity of an agent. The ionizing radiations inflict base damage, single and double strand breaks and complex lesion; but the repair of base damage and that of single strand is rapid. The ultra-violet light induces base damage mostly in the form of pyrimidine dimers, DNA-DNA cross links and DNA-protein cross links, while most of the lesions of the chemical compounds exist in the form of adducts bound to DNA by covalent bond. Each chemical might show a spectrum of additional sites and the kinetics of repair could vary considerably for each adduct site and also from cell to cell by a process of base excision repair. (iii) *The Drosophila test*—this organism might permit the total assessment of the spectrum of genetic effect while in other test systems, only one class of genetic damage can be studied at a time. The availability of various strains of *Drosophila*, known markers, might make one and the same experiment reveal the full spectrum of genetic changes from small deficiencies to translocations, lethal to visible mutations, mitotic to meiotic recombinations, etc. (iv) *Mammalian test systems*: Laboratory mammals, specially mice[12], because of their relevance to man, have been subjected more for the mutagenicity tests. Besides the host mediated assay mentioned before the mammalian models have been deployed for (a) *The micronucleus test* (MNT)—the quickest *in vivo* screening procedure for the detection of freshly induced structural chromosome aberrations in the bone marrow of the experimental animals. Due to the impairment of the spindle structure, the asymmetrical exchanges and anaphase laggards in erythroblasts undergoing their last chromosome replication and mitosis are set aside from the main nucleus; and they form micronucleus. They are often scored after the expulsion of the main nucleus. (b) *The sister chromatid exchange* (SCE)— using a pulse exposure of tritiated thymidine during S phase, Tayler[18] demonstrated by autoradiography in the next mitosis of such cells (M2), differentially labelled sister chromatids due to symmetrical exchange; but the recent BrdU-type techniques for SCE analysis have extensively been deployed to characterize not only the mutagenicity testing of different agents but also in the detection of locus specific effect and the chemical events associated with SCE. (c) *The bone marrow chromosome aberrations*— the superiority of mouse bone marrow as a means of testing clastogenic agents has been reviewed by Manna[12] and mentioned before. In recent years the different banding techniques for chromosomes have been deployed as an additional support in detecting more precisely the aberration point in bone marrow and cultured cells of mammals and man. (d) *Point mutation in mammalian cell culture*— Besides the common practice of studying the chromosome aberrations induced by different mutagens, in the cultured cells of different mammals and man, a method has been developed for detecting point mutation as a means of testing mutagenicity of different agents using mammalian tissue culture.

In the mutagenicity testing the positive effects obtained in somatic chromosomes and cells might imply somatic mutation leading to cancer but the detection of the effect in the germinal cells and on the progeny would have a direct bearing on the hereditary changes likely to be caused by the agent. Thus in the mammalian

systems, specially in mice, different methods of testing the germinal cells and related aspects have been developed. (e) *Mammalian male germ cell cytogenetics*—Though not a screening method for mutagenicity testing, the study of spermatogenesis time-table yielded differential sensitivity of spermatogonia to mature sperms to the effects of different mutagens. (f) *The heritable translocation test*—some mutagens treated to a male or a female or both parents might induce several types of translocation, of which the heritable reciprocal translocation has been adopted as the end point in the test. It might also affect fertility, reduce litter size etc. (g) *The dominant lethal test*—it is a very crucial test in which the zygotic death is assessed at different periods of gestation and after parturition for mating of the treated parent. The terminology has been open to question. It has been suggested for its replacement by the term 'lethal', because similar lethal effect has been found by us in F_2 for mating of F_1 individuals of the treated male parents mated to virgin females using chloramphenicol, D-glucoseamine hydrochloride and spores of *A. fumigatus*[12,19]. (h) *Abnormal sperm head morphology*—the sperm head morphology claimed to be under genetic control was found to undergo changes in count of sperms with abnormal head morphology in the treated males as compared to that of control. The method might serve as an additional evidence of the mutagenicity potential of an agent.

Besides several common methods for testing mutagenicity referred to above, many other methods like the use of insect cells other than *Drosophila*, higher plants, tumour cells, multiple loci method in mice, cytogenetic analysis of female oocyte of mammals, premature chromosome condensation, numerical sex chromosome anomalies in mammals etc., have also been advocated. Therefore, the development of these mutagenicity testing methods mostly around 1970 could be taken as one aspect of the new horizon of mutagenicity study. The other aspect has been the discovery and extension of the study of mainly the chromosome aberrations induced by living mutagens dealt later. Anyhow, in mutagenicity testing, results, applying more than one system are preferred now. The testing systems should permit (i) the analysis of the gene and chromosome mutation in germ and somatic cells, (ii) the evaluation of the chemical and its metabolites, (iii) the minimum experimental variability, (iv) the evaluation of dose-effect relationship etc. There are two categories of testing, (a) the screening system and (b) the complete system. The screening system includes the test on microorganisms with mutable activation *in vitro*, or test on *Drosophila* and the cytogenetic analysis of the bone marrow, while the complete system includes the test on microorganisms with metabolic activation *in vitro* and *in vivo* or the test on *Drosophila*, the dominant lethal test on mammal, the cytogenetic analysis of the bone marrow, and meiotic cells of mammals, the cytogenetic analysis of the effect on the culture of human lymphocytes etc. All these are just to avoid loose claims.

As the cytogenetical study of the living mutagens happened to be the latest addition in the field of mutagenesis mentioned before, the data in some cases are still scanty to establish them as mutagens on the same strong footing like some physical and chemical mutagens. Anyhow infectious diseases are known to occur from time immemorial and their detection and pathogenicity treatment took place later, while their mutagenic property to host and other treated cells has been the new horizon of the present discourse, the importance and implication of which has just begun to be assessed, though focussed earlier. The present state of knowledge of the effect of living[1,2] mutagens has briefly been depicted hereunder, which comprises microbes like viruses, ricketssia, mycoplasma, bacteria and pathogenic bacteria.

An universally acceptable classification of microbes is lacking. According to some, they are classed as (1) Microtatobiotes comprising two orders—Virales and Rickettsiales and (2) Schizomycetales bacteria comprising five orders— Actinomycetales, Spirochaetales, Mycoplasmatales, Eubacteriales and Pseudomonadales; of these chromosome aberrations, induced by any member belonging to Spirochaetales and Actinomycetales have not been docu-

mented so far, though a number of antibiotics obtained from *Actinomyces* has been found to induce chromosome aberrations in treated man and mammals[19].

Viruses, metaphorically called the naked gene, exclusively parasitic, simplest and smallest, among living organisms have the genophore either of DNA or of RNA but not both. Among DNA containing 6 major families, Poxoviridae, Herpesviridae, Adenoviridae and Papoviridae (oncogenic), and, among 10 RNA containing major families, Togoviridae, Picronaviridae, Reoviridae, Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae and Retraviridae (oncogenic), and among miscellaneous groups in which the DNA or RNA containing issue not settled, the Heptitis viruses were found to induce somatic chromosome aberrations in the cultured cells of the patients; and cells infected with different viruses, both in man and mammals but very rarely on other animals[2,20]. The aberrations were neither restricted to one specific group nor characteristic to DNA or RNA viruses and the damages caused by them were possibly due to their action on cell metabolism of the treated specimens. The effect was claimed to be delayed in appearance. Only the somatic chromosome aberrations induced by viruses have been studied so far. These could be generalized as individual ones, localised in the form of mainly chromatid type breaks, gaps, acentric fragments etc; and gross types involving the whole plate as diffuse or disintegrating forms like pulverization, pycnosis, stickiness and the numerical alteration like polyploidy and aneuploidy. The virus induced genome mutation could be the genome selection, while the similarity in the aberration types with that of antimetabolites and DNA base analogue induced ones, has been argued for their relevance to carcinogenesis. Further, the teratogenic effects were shown specially for the infection of rubella and cytomegalo-viruses, in which the faetal structural anlage was possibly altered, due to the virus-associated somatic mutation, if not by way of the viral cytopathic effect. Unfortunately, besides the somatic chromosome aberration study in mammalian models, only the dominant lethal test in *Drosophila* was carried out but opinions differed about the result. No other mutagen testing method has been applied so far, for which a definite claim of virus as mutagen remains open for confirmation. The virus induced bacterial transformation could support the mutagenic potentialities of viral agent.

Rickettsiales are the parasitic pleomorphic rod-shaped coccoid organism, larger than viruses and their genophore (chromosome) contains both DNA and RNA, with independent respiratory and synthetic capacity and resemble gram-positive bacteria and the causative agent of epidemic typhus. They had the background of changing history, as the French army could occupy Prague due to mass death of defenders; Charles the First abandoned his plan to march on London for fear of epidemic typhus. Among three genera, *Rickettsia grylli* were reported to induce chromosome aberrations in the gryllid insect but no study has ever been made on mammalian model and therefore, their mutagenic property remains open to question.

Among lower bacteria besides Mycoplasmatales, the chromosome breaking activity of species belonging to Eubacteriales and Pseudomonadales has almost exclusively been studied by us[1,2,12]. Mycoplasma or PPLO indigenous lower bacterial flora of the orthopharynx and genito-urinary tract of birds and mammals are prokaryotes lacking a cell-wall with body bounded by triple layered membrane and changeable shape. The chromosome aberrations in male meiotic cells of infected insects, *Nepa* and *Acheta* were originally discovered by Halkka and Heinonen[9] followed by the study of somatic chromosome aberrations, like virus induced ones in cultured cells of mammals and man. However, besides the clastogenic effect induced by mycoplasma no other mutagen testing method was applied and therefore, their mutagenic property remains to be established like that of viruses and rickettsia.

Among Eubacteriales, the log culture, culture filtrate and bacteria in saline suspension of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Strefaecalis*, *Klebsiella pneumoniae* and *Neisseria meningitidis* and log culture of

Salmonella typhi and *paratyphi* when intraperitoneally injected into mice caused variable degrees of bone marrow chromosome aberrations in the form of chromatid type breaks, constrictions, gaps, etc. as individual types and some grossly affected metaphases. The heat-killed sample of most of these bacteria induced hardly higher frequency of aberrations than that of controls while the toxin, of gas gangrene and cholera induced significant increase in the aberration frequency. The treatment of the bacterial samples indicated that the chromosomal effects were time-dependent, dose-dependent and nonrandomly distributed within and between the chromosomes and had retardation effect on mitosis except *Klebsiella*. Further, the fish pathogen *Micrococcus* sp. and the nonpathogenic *Sarcina lutea* were also shown (Manna 1982) to induce basically the same types of chromosome aberrations in bone marrow cells of treated mice. Among all these bacteria, *S. aureus* induced higher frequency of chromosome aberrations in regenerating the liver cells of partially hepatectomized mice and in the different stages of male meiotic cells. Further, among Pseudomonadales, the chromosome breaking activity in the bone marrow cells of mice treated with the culture of comma bacillus, *Vibrio cholerae* the causative agent for asiatic cholera, *Pseudomonas aeruginosa*, human skin pathogen and *Pseudomonas* sp. tumerogenic agent in fish was shown by Manna[2,12]. The aberration type was the same as that of the other bacteria studied by us. Further, the effect of *Pseudomonas* treatment revealed that it was tumerogenic as evidenced in fish, teratogenic as evidenced by the deformities of tail and other organs in F_1 embryos and progeny after birth from the treated parents, and mutagenic as evidenced from the high frequency of chromosome aberration in treated specimens, as well as in F_1 embryos of 15 day gestation, and individuals after parturiton at different age, higher frequency of abnormal sperm head morphology in male parent and progeny, and dominant lethals. It was of great interest to note that even in F_2 generation of the treated line, the frequency of somatic chromosome aberrations, abnormal sperm head morphology and lethals was higher than that of control line. Therefore, it has been suggested that all these bacterial species are potential mutagens because when more than one test system was applied for the treatment of *S. aureus* or *Pseudomonas*, the results were positive. It would also help to generalize that most viruses are also mutagenic agents. As data of ricketssia and mycoplasma are limited we make no positive claim.

The limit of horizon of the living mutagens is yet uncertain and it has been found not restricted only to prokaryotes but also extends to eukaryotes as the spores of *Aspergillus fumigatus* (belonging to the Class Fungi Imperfecti characterized by the lack of sexual stage and consists mainly of pathogenic moulds, yeasts, yeast-like fungi) could produce mutagenic effect in mice. This class stands in between bacteria and the fungi proper, and the family Moniliaceae contains some highly pathogenic genera *Aspergillus*, *Penicillium*, of which the mutagenic effect of the latter, though not tested directly, yet the antibiotic penicillin was proved to be clastogenic when experimented on mice[19]. The *Aspergillus* contamination degrades food material and the metabolites produced are collectively known as mycotoxins (aflatoxins, rubratoxins, ochratoxin etc.). Among them the chromosome breaking effects of citrinin on rat, Chinese hamster and human cells,and patulin on Chinese hamster cells have been documented while aflatoxin B^1 specially has been found to be, not only positive chromosome breaking agent in treated plant *Vicia* and *Allium*, and rodents and man, but also yielded positive results in other mutagenicity tests as liver microsomal assay of man or rodent, agar diffusion assay, DNA repair assay, in the protozoon *Chlamydomonas*, other microbial test methods deploying *Salmonella*, *E. coli* etc, heritable chromosomal changes in spermatocytes of treated males and in F_1 offspring, dominant lethal assay etc. Thus aflatoxin B_1 showed strong antimitotic, clastogenic, teratogenic and mutagenic properties in mammalian models like that of the

bacterium *Psuedomonas* sp. and the fungus, *A. fumigatus* tested on mice[12]. *A. fumigatus* can produce various toxins and antibiotics but not the aflatoxins. We found[2,12] that the introduction of the cultured spores of *A. fumigatus* into mice induced bone marrow chromosome aberrations like those of *Pseudomonas* within a few minutes to months together, breaks were nonrandomly distributed between and within the chromosomes, the effect was dose-dependent, it caused mitotic retardation, the count of sperms with abnormal head morphology was higher, higher frequency of dominant lethals, some F_1 embryos and individuals had higher frequency of chromosome aberrations etc. All the more it was also found that in F_2 generation of the treated line the lethal effect and the chromosome aberration effect continued in lower frequency. We also found that the treatment of the spores of *A. niger* and *A. flavus* when injected into mice, caused higher frequency of chromosome aberrations suggesting that all the pathogenic species might have the mutagenic property.

CONCLUDING REMARKS

In the foregoing brief review it has amply been indicated that some pathogenic living organisms mostly prokaryotes and some eukaryotes could act as mutagenic agent in their natural host or when introduced into other animals. Even similar effect was observed when a non-pathogenic bacterium *Sarcina lutea* was injected into mice. These observations widen the field of mutagenesis. The discovery of the living agents as mutagens might throw further insight into the problem of the evolution of the genetic systems and the genetic association leading at times to the genetic recombination of host-parasite characters. The host-parasite exchange of genetic elements by transduction, the occurrence of plasmid as extranuclear DNA, the behaviour of episome have been documented among microbes but similar possibilities could be guessed in bringing changes to the eukaryotic genetic system. The origin of the mitochondria from microbes is gaining support more and more on the association of bacterial gene in eukaryotes and the genetic marker of SV 40 has been suspected to be present in human genetic system. Though there remains a good possibility that all the microbes might not be mutagenic, a survey of this unknown world might reveal many mysteries of association of life and change of genetic elements. Thus while we have been progressing in researches on recombinant DNA in the laboratory experiments, it has already been taking place in nature screened by the Natural Selection.

The evidences in support of the living agents as mutagens are not so strong at present as that of physical and chemical agents because this avenue of research has been the latest addition in the field of mutagenesis but attention may be drawn to the fact that the physical and chemical mutagens exert their influence to bring change in the genetic get-up while the living mutagens in addition to the same role possess the potentiality of being incorporated at times *in part* or whole in the genetic system of the infected organisms specially in eukaryotes. Thus it may be mentioned again that though we have become somewhat alert about the environmental mutagens of physical and chemical nature, we are still utterly negligent to realize the utmost importance of the study of the living mutagens which are capable of inducing mutational change as much as that of chemical and physical agents. All the more the possibility of their incorporation in the eukaryotic genetic system is an added speciality as compared to the other groups of mutagens.

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Prof. B. V. Sreekantan, Director of Tata Institute of Fundamental Research, Bombay is the recipient of the second R. D. Birla Memorial Award of the Indian Physics Association, for his pioneering and sustained contributions in the field of cosmic ray physics. The first receipient of this prestigious award is Prof. Abdus Salam, a Nobel Laureate.

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