CHAPTER - 6

GENERAL DISCUSSION
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The aspiration of humanity to achieve a life of immortality, bliss and truth has been a burning flame that could not be put out by circumstances that seemed to deny it altogether. Many things have happened, many wild winds have blown which could easily extinguish the flame of this aspiration of the humanity. There were times when scepticism ruled the roost.

In one of the sacred scriptures of Hindu religion, the `Bhagvad Gita’ a discourse given by Lord Krishna to his disciple, Arjuna, Lord Krishna says

‘Jatasya hi dhruvo mrityur dhruvam Janma mritasya cha
Tasmad aparihariyarthna tvam sochitam arhasi’

‘For certain is death for the born and certain is the birth for the dead:
Therefore, over the inevitable you should not grieve’

It is the observation of very one that every living being that is born has to face death sooner or later. But what is important is that Lord Krishna seems to advise his disciple not to worry about the inevitable.

However, man’s quest for immortality has not stopped. Scientists with the hallmark of conquering nature could not stop wondering about the mystery of this inevitable phenomenon of aging and death.

Gerontological research all over the world appears to have two main objectives:

1) To understand the molecular basis of aging process
2) To circumvent or eliminate the debilitating physiological functions that are associated with old age.

In other words, one would like to see that the so called old age passes away in a reasonabli healthy way until the time of death. To conquer death,
which remains a remote possibility, does not seem to be the primary objective since it has its own physiological, social, cultural and philosophical ramifications.

As far as the molecular basis of senescence and aging is concerned, tremendous strides have been made. Several genes responsible for the onset of senescence have been identified and more on the way (Sugawara et al., 1990). In spite of the existence of several thoughts and hypotheses to explain the phenomenon of aging, there appears to be a consensus reached in that unattended or ill attended genomic damage appears to be the root cause of the many deteriorating physiological functions seen in old age. One of the key factors to maintain the genomic integrity is the DNA-repair process, which in itself perhaps operates in a genetically programmed manner. Indeed whether or not DNA-repair potential declines with age and its correlation with the aging process and therefore to the longevity has been a subject of considerable controversy and debate.

Some of the recent findings from other laboratories (Moriwaki et al., 1996) as well as those of the present investigation indeed demonstrate that basal DNA-repair capacity does decrease with age. In this unique study, perhaps first of its kind, a gradual decrease with age of the unscheduled DNA synthesis (UDS) in peripheral lymphocytes of normally aging individuals of Indian population, was shown (Please see the results in Chapter 3).

There has been a constant search among various laboratories not only for the biochemical and molecular biological parameters that are associated with aging, but also for those factors that could modulate the process of aging - particularly to delay the process.

In one of the significant observations in the field, Sugawara et al. (1990) have found that a gene or genes from human chromosome 1 can induce senescence in cultured immortalised cells. Similarly a couple of genes from
*Caenorhabditis elegans* were found to induce neurodegeneration (*Driscoll and Chalfie, 1991*).

It is important to note that while a number of genes / factors are identified as contributors to accelerated aging, so far no factor is discovered that can stop or delay aging process.

However, one factor that has been unequivocally shown to retard age-related changes is the restricted dietary calorie consumption. Ever since the original observation of *McCay et al.*, in 1935, much work has been done to show the beneficial effects of limited dietary consumption in many species although the same phenomenon is yet to be demonstrated in humans. These aspects have already been discussed in Chapter 3.

The present investigation provides some evidence to show that indeed reduced dietary calorie consumption for prolonged periods does show beneficial effects in terms of DNA-repair potential as seen in low BMI individuals. Secondly when the lymphocytes were stimulated by phytohemeagglutinin or challenged with UV light (254 nm), the response by way of enhanced DNA-repair parameters is always higher in LBMI group - particularly at adult and old ages - indicating the long term beneficial effects of low calorie consumption.

A weak link in the present studies is whether the low BMI individuals can be compared to those experimental models where dietary restriction was manipulated. In this connection, there are reports to correlate the BMI with nutritional status of the individuals (*James et al., 1988; Luzzi et al., 1991; Naidu and Rao. 1994*). The Indian Council of Medical Research (ICMR) through its established research institutes has conducted extensive surveys / studies and came out with data that correlate the BMI values with the nutritional status of the individuals. Based on these reports a BMI value between 16 - 18 is taken as mild undernutrition essentially due to calorie deficiency without any apparent clinical deficiencies and malnutrition. Extreme care was exercised in selecting the
Extreme care was exercised in selecting the subjects and this is the best one could perhaps do while using a naturally living human population as a model.

It has been a subject of great debate as to how dietary calorie restriction is able to achieve what has been reported in decelerating the aging process. McCay et al. (1935) hypothesised that slowing of growth and development was causal for the increased longevity of food restricted rodents. Another thinking is that there is a direct relationship between adiposity and mortality, and lack of excess body fat may be a cause for longevity. Another effect of calorie restriction appears to be reduced metabolic rate, thereby allowing the animals longer chronological period before total energy expenditure per gram tissue approached to that observed for ad-libitum fed rats (Sacher, 1977).

There is some evidence to point out that in rats dietary restriction results in higher rate of protein turnover through enhanced rates of polypeptide elongation (Wulf and Cutler, 1975). Since accumulation of altered proteins is one of the biochemical markers of aging, the increased turnover of proteins seen in diet restricted animals may not allow such accumulation of altered proteins, thus delaying the process of aging.

It may be logical to assume that when an animal is faced with limited amount of otherwise wholesome food, the whole metabolic machinery may be adapted to utilise the available energy for proper maintenance of soma. In such a situation there may be a trade off between the investment of calories for growth and reproduction and for the maintenance of the soma or adult body (Holliday, 1989). For the maintenance of the soma a variety of physiological / biochemical mechanisms may be essential while some other processes may not be so. Thus, the biochemical machinery pertaining to cell replacement, maintenance of genomic integrity of the cell are of paramount importance for the maintenance of soma. For the maintenance of genomic integrity, efficient and accurate DNA-repair is a must. Indeed this could be the reason for the observed
unaltered / improved DNA-repair parameters under calorie restricted conditions observed in earlier studies on experimental animals. The results of the present investigation support this concept and extend it to humans also.

Analysis of human mutations affecting aging may increase our understanding of the basis of genetic diseases. Concurrently we may also learn something about normal mechanism of the action of genes relevant to longevity and whether modifications affecting lifespan will be possible.

Martin (1977) has listed a number of genetic and chromosomal disorders that show signs of premature aging, apart from other symptoms. It is noteworthy that of the many syndromes that were listed Down syndrome appears to exhibit prematurely maximum number of features that are associated with aging. In addition, Werner's syndrome, Progeria, Ataxia telangiectasia and Cockayne's syndrome are also characterised by accelerated aging symptoms. Efforts to identify the locus of biochemical defect in these syndromes have revealed that the genetic abnormality in Werner's syndrome is mutation in a gene encoding a trans-acting factor that normally represses the production of an inhibitor of DNA-synthesis until an appropriate time at the end of replicative lifespan. Because of the mutation, the repression is ineffective and DNA-synthesis inhibited prematurely (Goldstein et al., 1990). In a more recent report (Yu et al., 1996) a gene that encodes a DNA-unwinding enzyme has been implicated with the accelerated aging seen in Werner's syndrome patients. In Ataxia telegiectasia the patients are found to have a defect in recognition and repair of γ-ray induced damage suggesting a reduced activity of endonucleases specific for this damage (McKinnon, 1987). A protein, named as ATM (Ataxia telangiectasia gene product) was identified most recently (Brown et al., 1997) and it is suspected that this protein may have something to do with fidelity of DNA-synthesis and cell cycle regulation following damage. Altered product of this gene may be responsible for aging characteristics of this syndrome.
It is indeed surprising that despite of the vast information available about the chromosomal aberration responsible for Down syndrome, information about the precise biochemical defect in these patients is scanty.

Despite the uniqueness of the aetiology of Down syndrome, there is really nothing unique either in the therapies and management strategies that are being employed or the means for preventing Down syndrome that are concurrently available to us. Stated another way, although we know that Down syndrome results from the presence of all or a part of a third chromosome 21 in the genome, we have not been able to capitalise on this knowledge either to prevent Down syndrome from occurring or to prevent or treat many of the components of the syndrome. Among the latter are, in particular, the developmental (mental) retardation, the increased frequency of leukaemia, the greater susceptibility to infection, and - perhaps of greatest concern in the long run - the development of Alzheimer disease.

The recent major advances in molecular biology, cell biology and genetics now make it possible for us to approach the problems of Down syndrome in entirely new ways, and the same holds true for advances in the neural and behavioural sciences as well. Things have never been more promising for developing an understanding of the causation and pathogenesis of Down syndrome and for using that understanding to enhance our ability to improve the physical and intellectual status of persons with this condition.

Chromosome 21 is small in size and one of the genes located on this chromosome and known to be affected is Cu-Zn superoxide dismutase. The levels of this enzyme are shown to be increased in these patients which may result in excessive production of hydrogen peroxide causing macromolecular damage. The few reports that are available on the DNA-repair capacity in Down syndrome subjects are contradictory. While decreased UV induced DNA-repair in fibroblast cultures (Rehborn and Pfeiffenberger, 1982) and leukocytes (Lambert et
of DS subjects was reported, no such impairment was noticed by Yotti et al. (1980). Chiricolo et al. (1993) have shown that DNA-repair after γ-radiation is actually enhanced and Zinc supplementation brings this to normal level.

The results of the present investigation show that all the uninduced DNA-repair parameters studied (Unscheduled DNA synthesis, activities of DNases, DNA polymerases, β and e) exhibit lowered levels in Down syndrome subjects, particularly in the age range beyond 25 years as compared to the age and sex matched controls. Also, a conspicuous age-dependent decline, particularly from 15 years of age onwards (compare the values of Group 2 to Group 3 in Tables 11, 13, 15, 17, 19, 21) in DNA-repair markers could be seen only in Down patients but not in normals. To our knowledge this is the first study of its kind dealing with DNA-repair capacity of Down subjects in some detail.

More important is the fact that while exposure to UV at a dose of 20 J/m² did show some response in Down subjects, the response did not increase with higher dose of UV (40 J/m²) but actually decreased. However, if the fold increase due to 20 J/m² dose of UV in certain parameters like UV DNase, AP DNase and DNA-polymerase e is considered, the values were either equal or even more than those of the corresponding normals (Tables 13, 15). These results are taken to indicate in Down syndrome that at the damage level induced by 20 J/m² of UV, there is some enhancement over the basal repair. However at higher dose of UV (40 J/m²) the cells were not able to elicit any further response. In fact the decreased fold increase with increased UV dose (Tables 14, 16, 18, 20) may reflect further damage to genomic structure in the absence of adequate repair arsenal.

Another significant finding of this investigation, in this author's opinion, is the manner in which the Down syndrome lymphocytes responded to MNNG treatment (See Tables 14, 16, 18, 20, 22 - Chapter 4). The response by way of fold increase, following treatment with this methylating agent, in the activity of
DNases and DNA-polymerases is similar to that found in normals except in the age group beyond 25 years (Group 3). Moreover, the basal levels of Polymerase e are significantly higher in Down syndrome at all the ages studied. It thus appears that polymerase e activity is spared very preferentially in Down patients and it would be interesting to probe into this aspect further.

MNNG is known to methylate the bases in DNA and thus induce mutations. It is now known that such alterations in DNA structures are repaired by base excision repair involving a short patch resynthesis with polymerase β and ε being implicated in such resynthesis (Singhal et al., 1995; Sobol et al., 1996; Mozzherin and Fisher, 1996). It will be therefore in line to speculate that in Down syndrome such repair processes requiring resynthesis of a short patch are spared at least to a reasonable extent while such DNA-damages like UV induced ones which require elaborate nucleotide excision repair involving resynthesis of a long patch are not repaired efficiently. This may well be one of the root causes for the accelerated aging seen in this disorder.

The average telomeric length at the ends of the chromosomes has a direct bearing on the ages of the somatic cell and its donor (a detailed account of this has already been presented in Chapter 5). Thus a longer telomere would indicate a chronologically younger age and the shorter one, the opposite. If low BMI individuals are indeed aging at a slower pace, as reflected by the DNA-repair parameters (Chapter 3), such individuals should also carry longer telomeres as compared to a normally aging person with adequate calorie consumption (the normal BMI subjects). The results reported in chapter 5, Table 24 do indicate that indeed is the case. Although this was a preliminary study and the sample size was small, still the data are quite pointing a 1 Kb longer telomere in LBMI subjects as compared to age matched NBMI subjects. This indeed would lend support and credibility to some of the conclusions drawn in Chapter 3 about the LBMI individuals being the undernourished and slowly aging population.
The converse must be true in the case of Down subjects. Once again, even with just two cases (Table 25) the trend is quite clear in that Down individuals show about 1 Kb shorter telomeres in their lymphocytes as compared to age and sex matched controls. This preliminary observation is in line with the observations of Vaziri et al. (1993) who found higher rate of telomeric loss (133 bp/year) with donor age in lymphocytes of Down patients whereas in age matched normals the rate of loss was 41 bp/year.

It is a matter of surprise that Down syndrome has not been used to adequate extent as a model to unravel the molecular biology of aging. The present investigation shows that one of the major and a fundamental process like DNA-repair is adversely affected in this chromosomal disorder. The rapid deterioration of DNA-repair potential with age and the possibility of the defect being inability to repair such damage which requires long patch resynthesis (e.g. UV induced damage) are the points to be picked up for further studies. It would be important and should be possible to identify the precise type of DNA-polymerase(s) and other enzymes / factors that are involved in long patch repair and to examine how they are affected in this syndrome.

Similarly the present studies also point out the beneficial effects of chronic but mild undernutrition even in human population. Such a model can once again be used to gain answers in the opposite direction i.e., which of the DNA-repair pathway that are preferentially retained or spared under the conditions. These further studies may well provide crucial answers as to the molecular events that may trigger or delay the aging phenomenon.