CHAPTER 1

INTRODUCTION
Ageing is a universal phenomenon. It is a characteristic of all multicellular organisms. By ageing we mean deterioration of the different organs and their functions after maturity of the individual. Birth, maturity and old age followed by death is an accepted fact of life. Until recently no serious efforts have been made by scientists to investigate the causes of ageing. However, the tremendous advances made in biological and medical research have succeeded in controlling some of the deadly diseases, thus decreasing the death rate of young people and increasing the life span on an average. In man, diseases like cancer and atherosclerosis which increase the frequency after the age of forty, when cured may prolong longevity by a few years only but cure of the ageing process may not only prolong youth and increase longevity, but may also defer the onset of all these diseases. Therefore, the problem before the scientists is how to keep the old people physically fit and how to make them useful to society. Here then arises the need for intensive research into the causes which lead to ageing.

The study of the problem of ageing is challenging. The important questions have confronted biologists are - Why do all organisms undergo deterioration of function after attaining reproductive maturity? Why do all members of a
species have a more or less fixed life span? At what age after attaining reproductive maturity does the process of ageing begin? Is there a trigger or switch which sets in motion the process of deterioration? If so, how is it switched on? Is this process programmed just as the process of development and differentiation are? Answers to these questions may help in devising methods to postpone or prevent the onset of the ageing process and thus control ageing. This would greatly enhance the work output in humans apart from giving them the psychological satisfaction of being young for a longer period. Prolongation of the youthful period would automatically increase the life span. But even if all the old age diseases are controlled, death is ultimately bound to come to the organism because it is a living system and like all other living systems is subject to the laws of nature. It will progress towards a more probable and equilibrium state with time and thus undergo gradual deterioration and death. Thus the motto of the American Association of Gerontologists rightly says 'Add life into years and not years into life'.

Though the life span of all animals of a species is fixed within a certain limit, different organs of an animal, however begin to show signs of ageing at different periods of its life span. It is not possible to define ageing in terms of a specific parameter. For convenience the chronological age (calendar age) of an animal is used as an index of its age.
Ageing is variously defined by workers in the field of biological gerontology. Shock (1961, '62), Weiss (1966) and Rockstein et al. (1977) consider ageing as the sum total of changes during an individual's life span, which are common to all members of the species or strain. Such a broad definition would normally include divisions of the life span of any organism into three trimesters: 1. embryonic development 2. maturation and 3. senescence. However, Rockstein et al. (1977) considered ageing as 'any time-dependent change which occurs after maturity of size, form or function is reached and which is distinct from daily, seasonal and other biological rhythms'. This presumably includes all of the post-maturational changes in an individual, including senescence. Comfort (1956) defined ageing as 'a biological process which causes increased susceptibility of an organism to disease'. According to Strehler (1971) ageing is a 'gradual decline in adaptation of the organism to its normal environment following the onset of reproductive maturity'.

The universal nature of the ageing process specially in bisexual animals empels the gerontologists to seek a laboratory animal which possesses not only the biological attributes of higher mammals, including man, but also those qualities especially suited to ageing studies. Among the large class of animals, the insects possess all such qualities. Insects include many species essentially domesticated, with similar temperature and humidity optima and with more or less
similar food requirements as man. Researches in the basic physiology and biochemistry of insects reveal that for the most part, their cellular structure and chemistry is essentially like that of their mammalian cohabiters of the earth. Indeed, the most observable differences between insects and mammals along such lines of investigation are no greater than between mammals and fish. Insects can be easily maintained under strict standardized conditions of diet, temperature and humidity at relatively little cost and a minimum of attention. Their availability in large numbers and with short life spans, they also have the additional advantage of having average longevities of several weeks to upto two months. They possess the multiple advantage of high reproductive potential and the availability of large number of offsprings in a single day, their short life spans permitting many replicate studies within short periods of time. All these criteria are suggestive of all the fact that the insects are the most suitable laboratory animals for ageing studies.

During the last twenty years three aspects of the problem of ageing in organisms have been recognized — (i) Biological, which includes studies on molecular, biochemical, physiological and structural aspect of the ageing process (ii) Clinical, which includes the study and cure of diseases common to old people, this is called geriatrics and is under the domain of the medical profession. (iii) Socio-psychological, which includes the study of the problem of old and retired people. These three aspects of the study together
are covered under 'Gerontology' (Geron = old man).

Modern gerontologists are studying the phenomenon of biological ageing in a wide variety of species, using a wide variety of techniques. At present more is known about the characteristics of ageing at the molecular, cellular, organ and system levels, than about the basic mechanisms involved. However, good working hypotheses are always based on a hard core of observations and are tested by making further observations. Many additional areas will surely have to be explored before the fundamental process(es) of ageing will be uncovered.

A tremendous variety of researches are being conducted giving different theories on ageing. Some of the important ones are as follows: Harman (1956) gave a theory of ageing based on free radical and radiation chemistry. Bjorksten (1958, '68, '80) is of the opinion that degenerative changes must have a basic cause on the molecular level. The possible rate of protein immobilization by means of progressive cross-linking reactions on potential cross-linking agents present in the bloodstream and of related physiological facts. According to cross-linkage theory of ageing, cross-linking is damaging to the tissues and involves loss of elasticity, reduced swelling capacity, increased resistance to hydro-lyses and probably enzymes generally, and thus an increase in molecular weight and a tendency toward embrittlement. There is a growing amount of direct evidence and much
indirect evidence for postulating the relationship between crosslinking and ageing. The theory that the progressive insolubilization and immobilization of proteins, nucleic acids and other large molecules is a principal underlying cause of age dependent degeneration, was proposed in 1941-42. Certain predictions made on the basis of the cross-linkage theory have been fulfilled. The crosslinkage is unique in meeting all of the criteria for a valid ageing theory (Bjorksten, 1980)

The general theory of senescence (Calloway, 1964) is the decline in efficiency of function with time of any chemical system without interference by the environment. As a matter of fact it is a decline in change in free energy of the total complex of chemical reactions involved in the maintenance of the chemical system. This decline may be viewed as a decline in storage of free energy as in endothermic reactions or it may be viewed as a decline in release of free energy as in exothermic reactions. This chemical system might be organic, inorganic, endothermic, exothermic, homogeneous, heterogeneous, molecular, ionic, atomic, nuclear or living cell. In all cases the system tends to go to the lowest possible free energy level for any given set of conditions of temperature, pressure, volume and concentration of reactants. In this way the living cell and hence all living thing behave according to this theorem since the only observable factors in the cell are matter and energy and
these follow all the usual pattern of energy-matter inter-
relationship (Calloway, 1964). Further considerations to-
wards an immunologic theory of ageing was discussed by
Wolford (1964, '69). The diffusion theory of ageing was
given by Carpenter (1965).

Hirsch (1974) gave the multistep theory of ageing in
a revised form. Accordingly he suggested that under certain
conditions the multistep theory yeilds the same expression
as the forbidden clone theory for the incidence of disease
as a function of age. Thus age-specific disease prevalence
data which has been advanced to support the forbidden clone
theory is equally supportive of the multistep theory. Dowd
(1975) explained ageing as exchange. According to him the
process of disengagement is the result of a series of
exchange relation in which the relative power of the aged
vis-a-vis their exchange partner increasingly deteriorates.
An imbalanced exchange ratio consequently results in which
the aged are forced to exchange compliance - the most costly
of all generalized reinforcers for their continued sustenance.
The retirement phenomenon is specified as illustrative of the
ageing as exchange process.

Felix (1975) gave a general hypothesis on ageing and
its relationship to the shortening of life of irradiated
organisms. According to him the collagen deterioration
theory is rendered questionable by the fact that doses of
radiation that shorten life do not accelerate the aggregation
of collagen. The progressive accumulation of lipofuscin, brown pigment granules, during ageing is related to the accumulation of cytoplasmic waste products. This ageing pigment accumulation in brown cardae atrophy possibly to a breakdown of lysosome activity.

Maynard Smith (1962) reviewing various aspects of senescence gave an account of causes of ageing. Last (1969) discussing the problems of ageing is of opinion that the ageing process is affected by numerous endogeneous and exogeneous factors. Bakst (1967) worked on prevention of ageing whereas Harman (1969) discussed the chemical protection against ageing. Berthauk and Beck (1975) reviewed ideas and present theories of ageing using chemical substances liable to interfere with ageing and developed the theory of so called antioxidizing substances. Emanuel (1975) describing the molecular mechanism and prospects for the prevention of ageing is of the opinion that disturbance of the free radical reactions play an important part in development of various pathological processes and ageing.

Kanungo (1975) discussing model for ageing takes into account two main characteristics of ageing viz. deterioration of adaptability of an organism to environment after reproductive maturity and determination of life span of a species. Differentiation, growth, maturity and senescence occur in this order and each of these phases has its own characteristic duration and speed in a species. This linear order occurs
due to sequential activation and repression of genes. Addition or deletion of any other changes of one or more genes in this sequence results in the origin of a new species, which may also accelerate or decelerate the sequence in the origin of a new species and thus decrease or increase its life span (Kanungo, 1975).

Curtis (1966) described the biological mechanism of ageing. According to Frolikia (1971) the reciprocal interaction between the three biological processes of regulation, adaptation and ageing determine the age-induced changes at the cellular level and the coupling of metabolic changes in the cell with shifts in neurohumoral regulation. Vladescu and Ieremia (1971) and Emanuel (1975) worked on some molecular mechanisms and prevention of ageing. Burch and Jackson (1976) giving a critical account of the molecular mechanism of ageing are of the opinion that two kinds of evidence, taken in conjunction, indicate that Orgel's theory is inapplicable to a wide range of disorders of sequence in man. The age dependence and the anatomical distribution of the lesions of such disorders imply a Burnet type forbidden clone theory of ageing.

Aspects of biology of ageing are being described by Clark (1967), Comfort (1968, '74) and Goldstein (1971). The literature on the subject of ageing was reviewed but no conclusions are presented by Comfort (1969). Goldstein (1971) is of the opinion that biological ageing may be a physiologic
process. According to him senescence is still consistent with high-biologic fitness in the evolutionary sense. Experimental gerontology attempts to explain the process which cause the human body to deteriorate with time, and to see whether and how the rate and character of this deterioration can be interfered when the deterioration is multiform, but its rate, as measured by the force of mortality, is highly stable (Comfort, 1974). Tschebotarew (1978) characterized the results of gerontological research in the USSR and discussed it long time development.

Cowdry (1952) and Strehler (1967) worked on ageing of individual cells and cellular ageing respectively whereas Little (1976) described the relationship between DNA repair capacity and cellular ageing. According to him ageing in mammalian cells may be related to a decline in the efficiency of normal DNA repair process. Researches on genetics of ageing and genetic factors associated with ageing are by Glass (1960) and Clark (1964) respectively.

Effects of nutrition on the life span and ageing in rats have been studied by Platt (1975). According to him the ageing process may be influenced directly by nutritional factors. Physiological studies on nutrition in old age and studies on the subjective significance of nutrition indicate that a training of nutritional habits in the sense of nutritional adaptation may be important in aged individuals (Schmitz, 1975). Ross (1969) described ageing in relation
to nutrition and hepatic enzyme activity patterns in the rat. Reviewing the literature on age differences in enzyme levels of mice, Florini (1975) concludes that differences in enzyme level do not provide a simple characterization of ageing processes.

Effect of age on the activities of enzymes and the concentration of nucleic acids in the tissue of female wild rats has been seen by Barrows et al. (1962). Klimenko and Shevtsova (1970) had seen the age-related changes in the DNA content and deoxyribonuclease activity in the liver of white rats. Herrman (1975) has seen the age-related changes in a spontaneously reassociating fraction of mouse DNA. Berg (1955), Everitt and Webb (1958), Ring et al. (1964) are specially mentioned here for their work on ageing in relation to blood and changes in erythrocytes of rats and Everitt (1957a) on the senescent loss of body weight in male rats.

The following data deals with the work done on human age-related researches by various workers. Shock (1961) wrote a review on physiological aspects of ageing in man and Comfort (1968) on the feasibility in age research on human beings. Stuchlikova et al. (1969) published changes of intermediary metabolism in obese persons and their relation to atherosclerosis and to primary cause of ageing. Winterer et al. (1976) has given an account of whole body protein turnover in an ageing man.
Rao and Patnaik (1972) worked on the ageing changes in the nucleic acids and protein contents of the liver of garden lizard *Calotes versicolor*. Gerking (1959) has seen the physiological changes accompanying ageing in fishes and Lukyanenko *et al.* (1971) saw that in the course of ageing in the white sturgeon, the total protein and albumin concentration increases. Studies on nematode ageing are very scanty and are done only in the last decade. Gershon (1970) studied nematode as a model organism for ageing research whereas Zuckerman (1974) saw the effect of procaine on ageing and development of a nematode.

In insects ageing has been studied by many workers taking different tissues. Workers like Hatai (1902), Ellis (1919, '20), Inukai (1928), Andrew (1938, '39) and Gardner (1940) have studied the neurons of higher animals during ageing and it has been reported that the neurons decrease in number as age advances.

Hodge (1894) studied the ageing neurons of honey bees and compared them with the ageing neurons of man. Smallwood and Phillips (1916), Pixell-Goodrich (1919) and Rockstein (1950) also worked on the ageing neurons of honey bees. Miquel (1977) studied the nerve cells degeneration in the brain of senescent *Drosophila melanogaster*. In *Musca domestica*, Rockstein *et al.* (1971) have worked on the age related neurosecretory changes. Sohal and Sharma (1972) worked on the age related changes in the fine structure and
number of neurons in the brain of the housefly *M. domestica*. Mishra (1980) worked on ageing of neurosecretory cells of *Dysdercus similis* and *Poekilocerus pictus*.


Regarding the accumulation phenomenon Haydak (1957) suggested that the age of bees can be best judged by the colour of their oenocytes. Wigglesworth (1950) and Fox (1953) have reported age dependent accumulation of pigments in the oenocytes and in the pericardial cells.

Accumulation of urates in relation to age as a storage excretion phenomenon has been described by Cuenot (1895) and Philiptschenko (1907a) for the fat body of the adult cockroach, by de Boiszezon (1930) for the adult mosquito (*Culex sp.*), by Metchnikoff (1915) for *Bombyx mori* and by Philiptschenko (1907b) for the insects which are without Malpighian tubules.

Hodge (1934) worked on the cuticle of honey bee in relation to age and Rockstein (1957) on age related changes in the wings of the honey bees. Blest (1960, '62, '63) worked on the flight behaviour in adult hemileucine saturniid moths in relation to age. Rockstein has done a lot of good
work on the biochemical aspects of flight muscles in insects. Rockstein and Gutereund (1961) worked on age-related changes in adenine nucleotides in flight muscle of male house fly. Rockstein and Brandt (1962, '63) have studied the biochemical basis for ageing of flight ability and enzyme changes in flight muscles correlated with ageing and flight ability in the male house fly. Bhatnagar and Rockstein (1964) have worked on physiological and morphological changes in the flight muscle of the ageing house fly *M. domestica*. Rockstein and Bhatnagar (1965) on age changes in size and number of the giant mitochondria in the flight muscle of the common house fly *M. domestica*. Rockstein and Srivastava (1967) on trehalose in the flight muscle of the house fly, *M. domestica* L. in relation to age, Rockstein and Baker (1974) on ageing of the thoracic flight muscle of the house fly *M. domestica*. Sharma and Jit (1981) have worked on wing abrasion with age in *Zaprionus paravittiger*.

Some workers such as Leob and Northrup (1917), Alpatov and Pearl (1929), Alpatov (1930) and Maynard Smith (1958) have seen the effect of temperature on ageing of *Drosophila*, whereas, Sharma et al. (1979) have seen temperature dependent longevity of *Zabrotes subfasciatus*. Jit and Sharma (1982) have seen temperature dependent variations in the life span of *Z. paravittiger*.

Effect of nutrition on ageing has been seen by Alpatov (1930) and Gardner (1948a, b) on *Drosophila* sp.
Haydak (1953) on cockroach, Rockstein (1959b) on *M. domestica*
and McCay *et al.* (1979) on male rats.

*Drosophila* has been worked out tremendously in
relation to ageing by various workers. To mention some of
these who did significant work on *Drosophila* are Maynard
Smith (1958, '62, '70), Sondhi (1964a,b), Clark and Maynard
Smith (1966), Harrison and Holliday (1967), Baumann and Chen
(1968), Hall (1969), Bozuk (1970), Burch *et al.* (1970),
Burcombe and Hollingsworth (1970), Hollingsworth and Burcombe
Samis *et al.* (1971), Wattiaux and Tesien (1971), Bozuk (1976)
Schmidt and Baker (1979).

In the last two decades, knowledge of the biochemistry
of insects has expanded considerably, becoming as important
as the chemistry of vertebrates and micro-organisms. Much of
the research into the process of ageing at cellular level has
been concerned with nucleic acids, proteins and the mechanism
of transcription and translation. These systems have been
extensively studied in mammals and changes in the amount of
these molecules present in the ageing organism has also been
studied.

During the last decade Frolikis (1972) has formulated
an adaptive regulatory theory of ageing, according to which
ageing is an internal contradictory process. The primary
mechanisms of ageing are related to changes in the control
of the genetic apparatus in regulatory genes, thus resulting both in qualitative and quantitative changes in protein biosynthesis which are the basis for the development of subsequent changes leading to ageing and death of a cell (Frolkis, 1972).

Alterations in the regulation of the macromolecular metabolism during ageing are now well recognized in the fields of DNA, RNA and proteins. These apparent biochemical manifestations of senescence were detected as alterations in physical properties of macromolecules, such as electrophoretic and chromatographic mobility, susceptibility to heat denaturation, structural resiliency and binding to other molecules, in intrinsic biological properties of macromolecules such as enzymatic activity in the magnitude and time course of stimulated rates of macromolecular synthesis and/or degradation etc. Unfortunately, the overwhelming majority of these studies have not progressed beyond the stage of phenomenology followed, in many instances, by premature theorization. It is not unfair to suggest that at one time or another each of the types of alterations probably attributed were, based on no conclusive experimental evidence to either deficiencies or gene expression or the accumulation of free radicals.

Ring et al. (1964) studied ageing and whole body metabolism. Biological role of enzyme changes in the ageing organism was studied by Bertolini (1964). Sinex (1966),


In view of the literature cited above on ageing in insects it is obvious that not much has been studied despite the large number of species in this group of invertebrates. The present investigation deals with the biochemical aspects of ageing in *D. similis* Freeman (Pyrrhocoridae; Gymnocerata; Heteroptera; Hemiptera). Biochemical aspects include both qualitative and quantitative analysis of proteins and quantitative estimation of nucleic acids in this insect during the process of ageing because alterations in the regulation of macromolecular metabolism during ageing are now well recognized in the areas of DNA, RNA and protein. For this study *D. similis* was selected mainly due to its short life span and availability in large number in areas around Sagar. Also it can be easily reared in the laboratory and is an insect of economic importance.

Numerous factors influence the life span of insects. Some are 'intrinsic', such as genetic constitution, sex, egg laying, others 'extrinsic', such as temperature, nutrition, population density and possibility to mate (Rockstein, 1973). Both intrinsic and extrinsic factors are often involved and it is difficult to relate time dependent changes in the constitution of an experimental animal to only one such factor. Nevertheless, the sum of time-dependent changes of physiological and other conditions in a single species may bring about an idea of what the 'syndrome' of ageing and senescence is.
Studies on insect ageing have indicated that there exists some correlation, both direct and inverse, between longevity and fecundity for female imagoes. In *Ephesia kuhniella*, Norris (1933) showed that virgin females live longer and produce fewer eggs than mated ones. There is an inverse relationship between egg production and life span in the cockroach *Periplaneta americana* (Griffiths and Tauber, 1942). Maurizio (1954) showed that worker bees in a queenless or broodless summer colony resembled in longevity the worker bees from overwintering hives. A greater longevity of virgin over mated females in *D. melanogaster* has been shown by Bilewicz (IN Comfort, 1956) and in *Drosophila subobscura* by Maynard Smith (1959). Virgin female hemileucine saturniid moths (*Automericia aurantiaca*) live longer than mated females (Blest, 1962).

Large differences in egg production and adult longevity have been reported for eight inbred races and one hybrid race of *D. melanogaster* (Gowen and Johnson, 1946). Here females of races that produce more eggs at a greater rate had a longer life span. This indicates that egg production is not of primary importance in the ageing process of these races. These results throw some question on the significance of the results of Bilewicz, quoted by Comfort (1956), and of Maynard Smith (1959), and, therefore, emphasize the need for critical reinvestigation of this entire problem.
Since the early studies on *Carabus* and *Drosophila* (Krumbiegel, 1929, '30) it is known that mating could have an influence on the life span of both males and females. The Author's intent in this study was to look for age dependent changes in RNA, DNA and proteins of *D. similis*, and for the influence of mating on these changes.