REVIEW
OF
LITERATURE
2.1 Ageing

Ageing is a complex phenomenon. The lives of all multicellular organisms begin with conception, extend through development, maturity, senescence and end in death. Ageing can be defined as a time-dependent process in which the body can no longer cope with environmental factors and changes as easily as it could initially (Makinodan, 1977), ultimately leading to an increased risk of dying. The ageing process is associated with deterioration at different levels of organization: the molecular, subcellular, cellular, tissue and organ levels, in addition due to the complex manner in which organs function. It is very difficult to differentiate between cellular and extracellular causes for functional impairment. A decrease in functional capacity with age can lead to disease. In fact, many diseases in ageing individuals might be complications of the ageing process. Mackay et al. (1977) distinguish different categories of age-related diseases in man, among which are autoimmune, neoplasia and vascular diseases. These diseases are characterized by degeneration and atrophy and infectious diseases.

Ageing must be the result of the side-effects of normal biological processes that are necessary to ensure the survival of the individual in the period of maximum youth and vigor. There is a positive correlation between ageing rate of a species and the mortality rate due to the hazards of its ecological niche. The rate of the ageing process is under genetic control to some extent for the manifestations of ageing, and life span differs between species and individual members.
of a species. Further, like all chemicals and chemical reactions, the manifestations of ageing which reflect chemical composition, and the rate of ageing process should be subject to environmental influences (Harman, 1981). According to Rattan (1989) ageing includes degradative changes in structure and function of an organism accompanied by many biochemical alterations at all levels of organization. The multicausal and multilevel nature of ageing process makes it difficult to pinpoint the fundamental causes of ageing phenomenon (Knook, 1991) (Fig. 2.1).

Medvedev (1990) reported that there are now more than 300 theories of ageing and the number continues to grow. Theories of ageing are commonly divided into "stochastic, programmed and evolutionary theories" (Sacher, 1968; Kirkwood, 1984; Holliday, 1986) or grouped according to the level of complexity of biological systems involved and listed as molecular and genome based cellular, physiological and organ theories (Shock, 1981; Frolkis, 1982; Hayflick, 1985). These are also classified according to the evolutionary system of classification, separating theories of ageing for different phyla in individual groups (Moment, 1982). The popular aspect theories include perturbations in the following biological systems or phenomena as a principal cause of ageing: the immune system, the neuroendocrine system, somatic cell mutation, the genetic program, error accumulation and repair selected physiological states, generation and scavenging of free radicals, cross-linking or instability of molecules, changes in entropy, lipofuscin accumulation and cell loss (Fig. 2.2).
Fig. 2.1. FLOW DIAGRAM SHOWING AGEING PHENOMENON.
Fig. 2.2. AN INTEGRATED THEORY OF AGEING IS SHOWN WHICH CONSISTS OF A COMBINATION OF OTHER MAJOR THEORIES OF AGEING. MANY OF THESE AGEING PROCESSES CAN RESULT FROM ONE ANOTHER.
Of all the theories of ageing physiological theories are most popular. They include (i) the free radical theory, (ii) the cross-linking theory, and (iii) waste product accumulation theory.

(i) Free radical theory of ageing

The most popular and widely tested theory is the "free radical theory of ageing" (Harman, 1956; 1981). Free radical reactions arise from (a) exposure of cells and their organelles to ionizing radiation, (b) nonenzymatic reactions, and (c) enzymatic reactions. Apart from these, the other sources of free radicals in biological systems are:

(a) Environmental sources - X-ray irradiation, light, air pollutants, insecticides, herbicides and drugs.

(b) Chemical sources - inorganic metals and organic - quinones, thiols, flavins etc.

(c) Biological sources - (1) non-catalytic proteins - heme proteins, (2) catalytic - membrane enzymes of mitochondria, microsomes, plasma membranes and soluble enzymes - xanthine oxidase, extracellular ceruloplasmin.

Harman (1981) emphasizes that the free radical theory of ageing is largely applicable to mammalian ageing, where $O_2$ is the main source of the damaging free radical reactions. Free radicals are highly reactive chemical species characterized by an odd number of electrons or by pairs of electrons of similar directional spin isolated singly in separate orbitals (Black, 1987). Molecular oxygen ($O_2$), though presents a unique
status for aerobic organisms, yet due to bi-radical nature it is prone to the formation of free radicals upon single electron addition. The major reactive \( \text{O}_2 \) species, superoxide anion \( [\text{O}_2^-] \), hydrogen peroxide \( (\text{H}_2\text{O}_2) \) and hydroxyl ion \( (\cdot \text{OH}) \) are derived from the mono-, di- and tri-valent reduction of \( \text{O}_2 \), respectively (Proctor and Reynolds, 1984) (Fig.2.3). The \( \text{O}_2 \) and its metabolites thus produced are potentially cytotoxic, clastogenic and deleterious (Richter, 1988; Simic et al., 1989). The cytotoxic metabolites of \( \text{O}_2 \) have been demonstrated to be involved in the per-oxidation of membrane lipids, consequently altering membrane composition morphology and function (Cavarocchi et al, 1986). Hidefuku (1984) hypothesized that free radicals may depress or inhibit the repair of sublethal damage of DNA which has been brought about by hyperthermia or enhance the lethal damage. The net effect of free radical reaction is irreversible and a major contributor to ageing (Richie Jr. et al., 1986; Segal, 1988). According to Harman (1981), the damage produced by the free radicals could be expressed as (a) cumulative oxidative alterations in collagen, elastin, and DNA itself, (b) breakdown of mucopolysaccharides through oxidative degradation, (c) accumulation of metabolically inert substances such as ceroid and age pigments by oxidative polymerization reactions, (d) changes in membrane characteristics of mitochondria and lysosomes and (e) fibrosis of arteriols and capillaries secondary to vessel injury by-products resulting from a peroxidation of serum and vessel-wall components.

Free radical damage can be thwarted by antioxidants such as \( \alpha \)-tocopherol, heme-containing peroxidases, selenium-containing glutathione peroxidase, superoxide dismutases and by DNA repair mechanisms. The
Fig. 2-3. Generation of free radicals and aim of free radical attacks.
major evidence for free radicals themselves is being modified. Dietary antioxidants increase life expectation in mice, rats, fruit flies, nematodes, rotifers and neurospora. According to Sohal and Allen (1985), a dynamic equilibrium exists between the rate of free radical generation and the antioxidant levels. The establishment of equilibrium is associated with differentiation, ageing and cancer.

(ii) The cross-linkage theory of ageing

The most venerable theory of ageing is that of "cross-linking". This theory focuses on molecular changes that are known to occur with age in substances within the extracellular compartment and, intracellularly, in information-containing molecules such as DNA and RNA. The importance of the extracellular compartment may be appreciated by observing that 23% of body weight is contained in the extracellular compartment (Kohn, 1978).

The theory holds that age changes result when two more macro-molecules become linked covalently or by a hydrogen bond. Such linkages, said to be reversible, accumulate over time. Molecular aggregation and immobilization increases, and the resulting inert or malfunctioning molecules accumulate and become increasingly resistant to catabolic processes. DNA may thus become damaged, leading to mutation or cell death. Irreplaceable molecules become reduced in numbers and intracellular transport may be impeded. Progressive reduction of space available for vital processes also occurs. Nonremovable cross-linked aggregates may "clog" glandular cells, thus impeding critical production and release of hormones and other cell products. Cross-linking in molecules such
as collogen could decrease solubility, elasticity and permeability. This would increase viscosity in the extracellular compartment, thereby impairing the flow of nutrients and waste products into and out of cells. These events are believed by the theory's advocates to result in all of the normal age changes observed at higher levels of organization. Cross-linking, they believe, is the first molecular event leading to most age changes.

At least for the collogen molecule an increase in cross-linkages over time has been demonstrated to almost every one's satisfaction (Verzar, 1956; Bjorksten, 1971; Kohn, 1978). There is also evidence for the occurrence of cross-linking over time in other proteins and in DNA (Bjorksten, 1971). Bjorksten (1971) lists an impressive number of naturally occurring known cross-linking agents to be found in living tissue: aldehydes, sulfur cross-linkages, alkylating and acylating agents, quinones, free radicals, antibodies, polybasic acids and their esters, citric acid, polyvalent metals, and cross-linkages formed as integral parts of reacting molecules such as conversion of specific lysine residues to aldehydes, which react to cross-link collogen. Bjorksten also argues that because free radicals are effective cross-linkers, the free radical theory is simply a special case of the more general cross-linkage theory (Bjorksten, 1974).

The cross-linkage theory has in its favour, first evidence that the phenomenon does indeed occur and, at least for some molecules, linkages do accumulate over time. Second the phenomenon has a known or theoretically probable, physiological basis, and finally, it is potentially universally applicable because the phenomenon occurs in molecules containing nucleic acids.
(iii) Waste product accumulation theory

The accumulation of waste products in the cell has been the basis for yet another theory of ageing. In this theory, it has been postulated that degradation products of cellular metabolism tend to accumulate with age and that the resultant cell mass tends to occupy more and more space in the ageing cell and thus mechanically interferes with the normal movement of metabolites within the cell and impinges on the living space of the cellular organelles. Lipofuscin for example is a pigment which is the byproduct of cellular metabolism and is composed of complete fatty acid derivatives (Tappel et al., 1973). Cephalin, sphingomyelin, cholesterol, esters, and lecithin have been found to form part of the composition of lipofuscin. The origins of lipofuscin have been postulated to be remnants of membranes or of mitochondria, but whatever its origin be, it is now generally accepted that the accumulation of lipofuscin increases with age. Vitamin E deficiency increases deposition of lipofuscin but supplementation of vitamin E does not inhibit lipofuscin accumulation or extend longevity. Others (Nandy and Bourne, 1966; Spoerri and Glees, 1974) report that centrophenoxine (dimethyl-amino-P-chlorophenoxyacetate) reduces lipofuscin concentration.

Although lipofuscin has been implicated in this theory of the accumulation of waste products, other substances have been investigated as well. Calcium and magnesium in particular have been thought to increase in concentration with increased age.
2.2. **Stress**

Literally the meaning of the word 'stress' is 'constraining force', but in various branches of science, it has different concepts and thus it has become a most complicated term. In the opinion of Yuwiler (1971), 'stress' like the word 'love' is broadly understood but poorly defined. He considered the life as a series of micro and macro-adaptations to a constantly changing internal and external environment and emphasized that stress was an everpresent condition. In the language of life-science, the meaning of 'stress' is "an intense force, strain, agent or mental condition which produces a defence reaction, which if continued or intensified, may lead to pathological lesions". According to Selye (1956), stress is the nonspecific response of the organism to any demand made upon it but this definition would not be satisfactory any more, because it is so wide that any physiological response and any external stimulus could be brought under this concept. Stress is a very divergent phenomenon. It was generally felt that there is a natural tendency to speak of a stress situation only when the demand exceeds a certain level and is experienced as undesirable or unacceptable. Rabkin and Struening (1976) said that stress like anxiety, was a broad and general concept describing organisms reactions to environmental demands. Selye (1976) further emphasized that stress causes certain changes in the structure and chemical composition of the body. Some of these changes are merely signs of damage, others are manifestations of the body's adaptive reactions, its mechanism of defence against stress. The totality of these changes, the stress syndrome, is called the "general adaptation syndrome" (G.A.S.) which develops in three stages.
(i) **Alarm reaction** (A-R) or shock, when the animal is initially exposed to stresses and must set up defenses to combat it.

(ii) **Stage of resistance** (S-R), when the organism is able to adjust to the changed environment. The adaptation is optimal at this stage.

(iii) **Stage of exhaustion** (S-E), in this stage the acquired adaptation is lost.

If we do not move all the way through these stages, stress gets bottled up inside a cumulative and insidious process (Fig. 2.A).

### 2.2.1 Various concepts of stress

Stress has different concepts in different branches of science (Fig. 2.5). Any environmental factor that can significantly modify an animal's biological responses resulting into stress is called a stressor.

#### 2.2.1.1 Physical concept

Physical conditions of the environment such as temperature, light and sound directly affect the internal environment of an organism, and create disturbances in the structural and chemical composition of the body. The resistance offered by the body against these conditions is termed as physical stress. If the organism has the capacity to adapt and combat these conditions, it survives, otherwise the death of the organism occurs.
Fig. 2-4 GENERAL ADAPTATION SYNDROME (G.A.S.)
Fig. 2.5 VARIOUS CONCEPTS OF STRESS.
2.2.1.2 Engineering concept

From engineering stand-point, the stress is resistance offered by the body to forces of deformation. According to Punmia (1972), "when a body is acted upon by external force or load, internal resisting forces are set up and it is then said to be in a state of stress". There are five types of stress:

(i) **tensile stress** - it exists between two parts of a body when each draws the other towards itself;

(ii) **compressive stress** - it exists between two parts of a body when each pushes the other from it;

(iii) **shear or tangential stress** - it exists between two parts of a body when the two parts exert equal and opposite forces on each other laterally in a direction tangential to their surfaces in contact;

(iv) **transverse or bending stress**; and

(v) **twisting or torsional stress**

2.2.1.3 Biochemical concept

Long-term studies aimed at throwing light upon brain mechanism of stress behaviour in biochemical terms are described by Consolo et al. (1965); Garattini et al. (1967); Valzelli (1967); Welch and Welch (1966, 1968); and Giacalone et al. (1968). These workers have focused their attention on the biogenic amine system.

Environmental stress invokes compensatory metabolic change through modification of the quality and quantity of enzymes that are normally
rate limiting or under fine control or inducible by hormones (Ramasarma, 1978). The processes of adaptation at the cellular level to chronic stress seem to occur by sequential changes in hormones, enzymes, and metabolites leading to a new steady-state. Ramasarma (1980) emphasized that those changes which are beneficial to the organism's need are promoted and accelerated. On long exposures, the changed pattern (due to stress) settles to a stage representing the delicate balance of cellular constituents best suited to the metabolic demands of the organism.

2.2.1.4 Physiological concept

Various theories (Selye, 1956; Mason, 1968; and Holmes and Masuda, 1973) have been postulated to elucidate the physiological response of the organism to stress, but none of them has been totally satisfactory (Burchfield, 1979). The most commonly accepted definition of stress as proposed by Selye (1956) is that it is anything which causes an alteration of homeostatic processes. Homeostasis, referring to the maintenance of the body's physiological resting state, is constantly changing in minute ways as a result of any behaviour, including sitting or yawning etc. Each movement activates physiological mechanisms which promote a return towards baseline. Physiologically, any type of postural change is considered as stress, and a stressor is the specific stimulus in the transaction. The 'stress response' is the organism's relatively nonspecific physiological response.
2.2.1.5 Medical concept

Stress, in its medical sense, is essentially the rate of wear and tear in the body. The feeling of just being tired, jittery, or ill are subjective sensations of stress (Selye, 1956). Mahl (1950) stated that prolonged exposures to noxious environmental stimuli are aetiological factors contributing to the development of certain disease states in man, such as coronary artery disease, gastrointestinal tract disorders, and hypertension. Rats subjected to chronic intermittent exposure to environmental stressors (auditory, visual, or motion) develop and maintain systolic hypertension (Smookler and Buckley, 1970).

Rabkin and Struening (1976) propounded that stress can be one of the components of any disease, not just as those designated as 'psychosomatic'. As Dodge and Martin (1970) have stated, "the diseases of our times, namely, the chronic diseases, are aetiologically linked with excessive stress and, in turn, this stress is a product of specific socially structured situations inherent in the organization of modern technological societies".

2.2.1.6 Social concept

"Social stressor" is the change in personal life, such as bereavement, marriage, or loss of a job, which alters the individual's social setting (Rabkin and Struening, 1976). Holmes and Rahe (1967) proposed a more specific definition. He defined "any set of circumstances, the advent of which signifies a required change in the individual's on-going pattern is termed as "social stressor"."
In the aetiology of various diseases, the role of stressful life has been a field of research for more than four decades. Sponsored by the Association for Research in Nervous and Mental Diseases, a formal recognition to the field on "Life Stress and Bodily Disease", was first given in the year 1949 after Cannon's early observations of bodily changes related to emotion and Adolph Meyer's interest in the life chart as a tool in medical diagnosis.

Following the Dohrenwend and Dohrenwend (1969) conceptulization, the perception of stressful events is mediated by two broad categories of variables, one consisting of personal or "internal" factors and the other of interpersonal or "external" ones. Personal factors include, for example, biological and psychological threshold sensitivities, intelligence, verbal skills, morale, personality type, psychological defences, past experiences, and a sense of mastery over one's fate (Wolf and Goodell, 1968; Dohrenwend and Dohrenwend, 1969; Rahe, 1974).

2.2.1.7 Psychological concept

Stress has also been regarded as the organism's response to stressful conditions called stressors, consisting of psychological reactions, both immediate and delayed. Psychological homeostasis (Engel, 1953) refers to the maintenance of the normal mood state of an individual at rest. All emotions are alterations from this state. What was commonly known as the "grief cycle" (Kubler-Ross, 1969) was really an adaptive mechanism activated whenever an organism was faced with a change (Falek and
Britton, 1974; Navaco, 1976). Thus the phenomena of denial, anger, guilt, depression and acceptance enable the organism to integrate the event into consciousness and to promote psychological adaptation. Schachter and Singer (1962) propounded that psychological changes were usually accompanied by alterations of physiological homeostatic responses. If alterations of psychological homeostasis are considered as stress, it is possible to differentiate between those events which cause alterations of physiological response but are psychologically stressful, and those which are not. Therefore, the psychological homeostatic responses include both conscious and unconscious states. In other words, perception of an event's occurrence is necessary if it is to be classified as a stressor. Once it has been perceived, it may be either integrated or defended against on a conscious or unconscious level. Under this definition, individuals classed as "repressors" (Scarpetti, 1973) or "deniers" (Wolff et al., 1964) can be distinguished from those classed as "suppressors" (Levi, 1972) or individuals who do not recognise the stimulus as stressful (Hinkle and Wolff, 1957). Whether the psychological and physiological homeostatic systems are acting parallel (Brady, 1975) or interactive (Wine, 1971), is not known. Schopenhauer (see Lajtha, 1971) remarked that "it is not what happens to a man that is important, it is what he thinks happened to him". In the same manner, it is not simply stress, but the kind and magnitude of the stressor, that alters life. Stress plays a role in such diverse manifestation of life as ageing, the development of individuality, the need for self-expression, and the formulation of man's ultimate aims. Psychological stress arises in man due to the effect of various factors on the neocortex. Experiments in animals have shown that deep and prolonged psychosomatic disturbances arise from psychological stress.
2.2.2 Physiology of Stress

It is generally agreed that the central nervous system is a necessary mediator of the pituitary responses to stress (Yuwiler, 1971). Physiological mechanisms underlying stress are identical in man and animals.

2.2.2.1 Hypothalamic-pituitary-adrenal interrelations

The hypothalamus was thought to be ultimately involved in response to stress by Ganong and Hume (1954). The contribution of other areas of brain is less clear, but the structures which are considered to bring changes in the pituitary response to stress are: hippocampus (Mason, 1958; Kim and Kim, 1961); reticular formation (Anderson et al., 1957; Endroczi and Lissak, 1960); limbic cortex (Knigge, 1961); and rhinencephalon, temporal and occipital cortex (Setekleiv et al., 1961). Guillemen and Rosenberg (1955), Saffran et al. (1955) and Schally et al. (1960) stated that the median eminence region of the hypothalamus is particularly involved in the control of ACTH production by the pituitary. They further expressed that the release of ACTH is controlled by another hormone or a set of hormones called the corticotropin releasing factor or CRF which is supposed to be secreted by neurosecretory cells in the supraoptic and periventricular regions of the hypothalamus. These discharge in the median eminence and are carried to the anterior hypophysis by a vascular network called the pituitary portal system. Further, it was stated that though CRF is necessary for mediation of pituitary ACTH release following some stressors, it may not be required for all.
Different hypothalamic sites may be involved in mediating different 'ACTH-induced' responses of the adrenal, such as the increase in adrenocortical hormone and the depletion of adrenal ascorbic acid under different stress conditions (Slusher, 1958; Nowell, 1959; Smelik, 1959).

Mialhe-Voloss (1958) reported that though anterior pituitary releases the major portion of ACTH, the posterior pituitary also contains an ACTH. It is not exactly known whether these two ACTHs are chemically identical, but there does seem some selectivity in their release. It has been demonstrated that some types of stressors, such as loud sound, selectively release ACTH from the posterior pituitary while most of the stressors release ACTH from the anterior pituitary (Rochefort et al., 1959).

ACTH excercises its primary effect on the adrenal cortex and appears to stimulate release of unesterified fatty acids from epididymal fat pads (White and Engel, 1959), to stimulate melanin formation and to induce pigment dispersion of melanophores (Hu and Chavin, 1956). Besides these effects, ACTH appears to elicit a number of behavioural effects. It is reported that ACTH causes delay in the extinction of conditioned avoidance response (De Wied, 1966; Bohus and De Wied, 1966), and when injected directly into CSF or brain ventricle, to produce sexual excitement (Bertolini et al., 1969) and a peculiar behavioural syndrome of stretching and yawning (Ferrari, 1958).
2.2.2.2  Adrenocortical hormones

Following any stressor, glucocorticoids (mainly, cortisol, corticosterone and possibly 18-hydrocorticosterone) are released rapidly into the bloodstream, reaching peak values in 15 minutes, but corticoid levels just as promptly return to baseline if the stressor was acute in its effect. Such would be the case with an injection of small volumes of saline, for example. On the other hand, stressors, such as fasting, cold, or laparotomy lead to more sustained elevation in serum corticoids.

There is a remarkable tissue specificity in the action of adrenocorticoids. A set of changes produced in one tissue may be quite opposite to those produced in another tissue. Curiously, little is known about the direct effects of adrenocorticol hormones upon the brain itself. Administered corticoids produce behavioural changes like abnormal EEGs, altered evoked potentials in the diencephalon, and blockage of spontaneous activity in periventricular grey matter of the third ventricle of brain (Gray et al., 1951; Kohn et al., 1961; Sambhi et al., 1965). Perhaps, the most dramatic effects of glucocorticoids on brain are the marked changes in the brain size and composition following treatment of the neonate with corticoids. A single injection of corticoid on first day of life markedly retards whole body growth and even more markedly affects brain size, particularly, cerebellum size (Howard, 1968a,b; Singh, 1988).

2.2.2.3  Adrenal catecholamines

If stressors are disturbers of homeostatic peace, adrenal corticoids act both as restorers of equilibrium and metabolic planners. Different
stressors obviously present different challenges and require different metabolic answers. Stimulation of sympathetic nerve endings releases norepinephrine: tyrosine hydroxylase, the rate limiting step in norepinephrine synthesis converts tyrosine to 3,4-dihydroxyphenylamine (DOPA) which is, in turn, decarboxylated to 3,4-dihydroxyphenylthylamine (dopamine) by 5-HTP-DOPA decarboxylase (Nagatsu et al., 1964; Udenfriend, 1966). Dopamine is transported to cytoplasmic organelles containing dopamine-3-oxidase which converts it to norepinephrine (Crout, 1966; Levin et al., 1960). In addition, some vesicles in the adrenal appear to contain the enzyme phenolethanolamine-N-methyl-transferase (PNMT) which methylates the free amine on norepinephrine to form epinephrine (Axelrod, 1962). Upon neuronal stimulation, both epinephrine and norephinephrine are released. Once released, the catecholamines are rapidly bound peripheral tissue, degraded or excreted, as either free or catecholamine (Douglas, 1966). Although many of the pharmacological actions of epinephrine resemble the physiological responses in acute stress, it is only in very extreme stress that epinephrine really contributes to these responses. In general, catecholamines then serve in acute stresses to prepare the organism for action, by activation of noradrenergic-sympathetic system and to provide the energetic resources to carry out such actions by the metabolic effects of epinephrine (Singh, 1988).

2.2.3 Stressors

Any environmental factor that can significantly modify an animal's biological responses resulting into stress is called a stressor. Yates
(1967) emphasized that all stressors cannot be visualised in the same light but rather are related to each other as games are related by a set of overlapping phenomenon in which all elements are not necessarily present in all members of the class. Rabkin and Struening (1976) opined that stressors of sufficient intensity and duration will induce a response on acute stress reaction in all so exposed, regardless of predisposition. The effects of stressors are species-dependent. Heat is a lesser stressor to a gerbil than to the polar bear. Stressors are usually considered as single entity. In the real world, stressors are combined as the physiological shifts required for adaptation to such combinations which may be quite different than the algebraic sum of the responses to the individual stressors.

2.2.3.1 Classification of stressors

Yates (1967) divided stressors in class I and class II stimuli on the basis of their ability to elicit ACTH release despite prior administration of the synthetic long-acting steroid, dexamethasone. According to Yates (1967), class I stimuli consist of stressors such as ether-burns, electric-shocks, and laparotomy in which ACTH release is blocked by prior corticoid treatment, and class II stressors are such as haemorrhage, intestinal traction, cervical dislocation and anoxia, in which blockade does not occur. Conceptually, stressors can be divided into (i) those which compromise the organism and require the restorative processes, and (ii) those which threaten but are harmless. Yates (1967) placed the real **Physiological stressors** of the natural environment, hunger,
thirst, infection, exhaustion, cold, heat, tissue-damage etc. in the first category; while the Laboratory stressors such as shock, shaking, immobilization or restraint and handling etc. in the second. Yates (1967) further stated that experimentally the distinction between the physiological and laboratory stressors is much harder to make, particularly in the case of the animal. According to him, humans in a stress-study on hunger, for example, are generally secure in the belief that death by starvation is not part of the experiment. The rat has no such assurance.

2.2.3.2 Restraint stress

When an animal is kept under control in such a way that it cannot move its body at its will it is said to be under restraint stress. For example, when a rat is kept in a mini cage (Hasan, 1985) with the result that its movement is restricted, it is said to be under restraint stress.

2.2.3.3 Manual restraint

Rats of most strains can be picked up manually and restrained (Baker et al., 1980). Some strains have acquired reputation for being particularly aggressive, while others are thought to be particularly docile. Baker et al., (1980) emphasized that proper manual restraint by a confident and firm grasp from above about the thorax with the thumb brought up behind the chin of the rat will prevent the animal from
biting under most circumstances. However, squeezing the neck and thorax must be avoided, otherwise the rat will react by struggling and possibly, biting.

2.2.3.4 Restraint devices

In the works of Renaud (1959), Baker et al. (1962), Ganis (1962), Thompson (1966) and Giradet (1974), details of many types of restraint devices, cages and other apparatus for rats, which are commercially available for general and/or specific needs, may be seen.

2.2.3.5 Restraint used as a stressor

Restraint is one of the commonly used laboratory stressors which has the apparent advantage of being relatively simple. It was further observed that this particular stressor is compounded of fear, isometric muscular activity, and muscle cramps due to specific positioning. According to Yuwiler (1971), restraint poses some problems since the magnitude of the stressor varies with experience and more subtly, with differences in positioning and handling the animals between experiments and experimenters.

Seegal (1981) advocated that immobilization is usually selected as a stressor or as a means of inducing stress because of its being simple and also due to the large number of studies that have employed this stressor (Kvetnansky and Mikulaj, 1970; Kobayashi et al., 1976; Reigle and Meites, 1976; Kholkute and Udupa, 1978, 1979; Du Ruisseau et al., 1979; Kawakami et al., 1979; Krigger et al., 1979).
2.3 Acetylhomocysteine Thiolactone (Citiolone)

Systemic name: DL - N - Acetylhomocysteine thiolactone.

Structural formula:

\[
\text{NH} - \text{C} - \text{NH}_3
\]

Acetylhomocysteine thiolactone belongs to thiol or sulfhydryl group (-SH) donor drug (Totaro et al., 1985). The -SH group plays an important role in detoxification in tissue. Protection of free radical by -SH group is well known. Compounds containing -SH group often prevent cellular transformation by oxidants, yet increases in cellular thiol status have frequently been observed in cells at various stages of transformation. Although it has been suggested that shifts in -SH status may play a causal role in transformation, it seems more probable that the increases in -SH status are associated with mitotic activity (Allen and Balin, 1989). Citiolone elevates neuronal sulfhydryl groups after chemical toxicity (Hasan and Haider, 1989). It also inhibits the lipofuscin pigment formation (Totaro and Pisanti, 1987).

To-date, no attempt has been made to evaluate the effect of citiolone in the various regions of the rat brain and spinal cord in aged rats following restraint stress. Therefore, it would be of special interest to evaluate the mode of action of acetylhomocystein thiolactone on different biochemical parameters.
2.4  Morphological Changes of the CNS

2.4.1  Gross Changes

Morphological or structural organization of the brain were related with physical alterations as brain weight, volume, convolutions, ventricular space and general atrophy with age (Bondareff, 1959; Andrew, 1971). The aged brain of higher mammals, primates, canines and humans showed loss of brain weight, reduction of brain volume, increase in the ventricular size and the size of the sulci (Brody, 1955; Brizzee, 1975; Adms, 1980). The decrease of brain weight occurs mainly because of dry residue and to a lesser extent water loss. Samorajski and Rolston (1973) showed a more marked water loss in the brain stem and a less marked water loss in the hemispheres and cerebellum of mice. Studies of the age-related changes in cerebrum and cerebellum individually have shown that each of these two major components of the brain decrease in weight with age. The brain undergoes a period of very rapid growth during perinatal development, remains stable throughout maturity and then appears to decline in mass during senescence. However, in some rodents (rats) the brain weight in old age increases or remains unchanged as compared with adult age, which could be due to its lower weight, but they do show a decrease in the neuronal density (Bondareff, 1980; Casey and Feldman, 1985).

2.4.2  Anatomical changes

The neuroepithelial cells of the neural tube give rise to the neurons, astrocytes, oligodendrocytes and ependymal cells that largely constitute
the central nervous system (Jacobson, 1978). The normal ageing brain exhibits both qualitative and quantitative changes in (1) neuron number, (2) dendritic extent and (3) synapse number and structure. These anatomical changes probably relate to decrease in both behavioral capacity and neuroplasticity associated with ageing. There is a decline in cell number within the cerebral cortex during normal ageing, along with a decrease in overall cortical volume (Coleman and Flood, 1987).

Dendritic extent measured at any one point in time reflects the simultaneous, ongoing processes of growth and regression (Flood and Coleman, 1990). Synapses are dynamic and can be remodeled in response to both external and internal cues. However, this neuroplasticity may or may not be adaptive. For instance, dendritic regeneration may represent a loss of critical synapses or focusing of synaptic activity which serves to provide a more rapid or efficient response to environmental stimuli. Sprouting, on the other hand, has sometimes, but not always, been suggested to underlie functional recovery. For instance, the contralateral sprouting of septal afferents following entorhinal cortex lesions may occur in the absence of behavioral deficit or at a time without relation to the lesion-induced behavioural impairment (Finger and Almli, 1985). Analysis of cortical tissue taken from cognitively intact aged humans has repeatedly demonstrated an age-related increase in dendritic extent despite cell loss (Buell and Coleman, 1981; Flood et al., 1987). Therefore, the ageing brain might compensate for neuronal loss by extending its dendritic territory.
Synaptic structure has also been shown to exhibit changes over the lifespan. During early development in rodents, there is a thickening of the postsynaptic elements which undergo steady, nearly linear increases in length, area and average thickness in early development and then stabilize for the remainder of the lifespan. Similarly, the length, area and thickness of the presynaptic element increases during early development, then plateaus, with no significant alterations even through old age. In the light of these findings, ageing in the rodent might be characterized by few quantitative alterations in synaptic element structure, but an overall decrease in synaptic density which accompanies declines in neuronal density (Hasan and Glees, 1973b). Furthermore, McWilliams and Lynch (1984) report that aged rats exhibit virtually no synaptic replacement in the hippocampus following damage to commissual pathways. This is in contrast to the extensive synaptic reorganization observed in young and middle aged rats following similar lesions.

These changes could account, in part, for both physiological and cognitive deterioration. First, aged organisms exhibit a loss of neuron density, notably in the cortex and the hippocampus areas known to be involved in attention, memory, and other higher cognitive functions. There is an accompanying decline in the number of synapses present in these areas, though remaining synapses appear to be structurally stable. Aged tissue fails to exhibit a normal growth response following neuronal loss. Taken as a whole, these alterations suggest one aspect of brain ageing which is a decreased ability to respond adaptively to physiological and environmental stimuli, on both a cellular and systems level.
2.4.3 Light and electron microscopic changes

In Purkinje neurons of the cerebellum, pyramidal neurons of cerebral cortex and trigeminal ganglion cells, distinct nuclear differences have been reported between young and old animals in light microscope preparations. In these studies, the differentiation of the nucleus from the cytoplasm was very sharp in young animals but less so with advancing age and in old animals. According to Brizzee et al. (1975), the same tendency occurs in human tissues but individual variation appeared to be greater. They believed this decrease in differentiation between nucleus and cytoplasm with age to be due to a greater basophilia of the nucleoplasm in cells of old subjects and to a decrease in amount of Nissl substance in the perikaryon. The nucleoli in such cells were often paler than in cells of young animals. Some investigators have described an increasing darkening of the nucleoplasm and accumulation of Feulgen stainable chromatin material with age (Sanides, 1957; Klatzo et al., 1965). Increased nuclear basophilia with vacuolization of nucleoli in spinal cord neurons of rats has also been described (Andrew, 1961). Oval or round enlargements (spheroids) of axons in the tissue from ageing individuals have also been reported. These exhibited marked variation in the nucleus gracilis. They have also been observed, though to a lesser degree, in other brain stem structures and basal ganglia (Brizzee et al., 1975).

According to ultrastructural studies of age differences in neuroglia in the rat hippocampus, there is increased oligodendroglial satellitosis, binucleated cells or fusion of oligodendroglia and invagination of oligodendroglial nuclear membranes with a marginal condensation of nuclear
chromatin with age (Hasan and Glees 1973b). Obvious changes in astrocytes were not observed, but in microglia, numerous lipid vacuoles have been observed in the perikaryon. Increasing accumulation of neurofibrils and neurotubules and higher electron density of axons has also been reported with age (Hasan and Glees, 1973b). Age-related changes in the rat hippocampus have revealed a disorganization of the normally well-developed laminated pattern of the rough-surface endoplasmic reticulum and multiple invaginations of the nuclear membranes with age. In old rats, the outline of the neuronal nuclear membranes typically appears less spherical than in younger animals, showing more distortion and infolding (Hasan and Glees, 1973b; Johnson and Miquel, 1974; Vaughan and Vincent 1979; Johnson, 1980). Increase in filament-filled glial profiles coursing through the neuropil were also reported in aged rats (Vaughan and Peters, 1974). In the ageing simian brain there is an increase in the number of synaptic processes containing membrane-bound degenerative products and dense osmiophilic bodies (Mervis et al., 1979). Wisniewski et al. (1973) reported a similar accumulation of debris in the synaptic processes of aged monkeys. The actual presence of this material may not have any age-related significance, as similar degenerative products in altered axonal terminals have been found in the lateral vertibular nucleus of normal adult rats (Sotelo and Palay, 1971) as well as in ageing rats (Johnson and Miquel, 1974). Spontaneous axonal degeneration has likewise been found to be more severe in the ageing rat brain than in younger animals (Johnson and Miquel, 1974). Disruption of the myelinated fiber may involve both the axon and its myelin sheath or myelin degeneration surrounding a normal axon. In rats (Sotelo and Palay, 1971) and man
(Rees, 1975), axonal-myelin alterations have been observed in normal young and adult brains. The phenomena, therefore, may be manifestation of normally occurring axonal remodeling as well as an age-related dystrophic finding.

Lipofuscin deposition exhibits a special structural, topistic and qualitative appearance as well as a special developmental course in the ageing processes of the nervous tissue. The increasing intraneuronal accumulation of lipofuscin pigment is considered to be one of the consistent cytological alterations correlated with mammalian ageing (Bondareff, 1957; Brody, 1960; Nandy and Bourne, 1966; Reichel et al., 1968; Samorajski et al., 1968; Brizzee and Johnson, 1970). According to Strehler et al., (1959), the cause of lipofuscin accumulation must be seen in the difficulties affecting the elaboration of a sufficiently stable membrane for containing lysosomal enzymes. These enzymes are readily liberated during cellular reorganization leading to lipofuscin formation in relation to ageing. The lysosomal origin might also apply to neuronal lipofuscin formation. That the accumulation of pigment is a 'normal' age-correlated phenomenon has not been generally accepted, for Sulkin (1969) pointed out that lipofuscin might be the result of various forms of insult and injury to the organism. Pigment formation in young rats has also been found in a number of experimental conditions such as chronic vitamin E deficiency (Einarson, 1953, 1954; Sulkin and Srivanij, 1960), the effects of acetanilid feeding, the treatment of unilateral nephrectomized rats with ACTH and in cases of chronic hypoxia (Sulkin, 1969). Sulkin and Srivanij (1960) stated that environmental, emotional and physical stress and not age per se could
initiate the deposition of lipofuscin cells. Strehler (1962), however, contends that experimentally induced lipofuscin need not invalidate close relationship of the pigment with ageing since the resulting experimental changes occur by a different process than that in physiological ageing. According to Reichel et al. (1968), the amount of lipofuscin also increases with age and is distributed in the brain in a highly selective manner. The relative amount of pigment has been considered to be a fairly reliable index for the chronological age (Nandy, 1968; Reichel et al., 1968). Nanda and Getty (1971), examining lipofuscin pigment in the nervous system of ageing pig, proposed that the presence of lipofuscin pigment was the only neurocytological age alteration in the neurons. The presence of pigment in all of the old animals, the increasing rate of deposition with advancing age, the marked presence in nondividing cells and the lack of correlation with any specific disease are strong arguments supporting its relationship with the ageing process (O'Steen and Nandy, 1970). Some of the earlier reports (Hamperl, 1953; Bachmann, 1953), which state that the amount of lipofuscin in the organ may decrease with time, should be mentioned here. In the absence of quantitative data in support of the above, it is difficult to evaluate the consensus of opinion that increasing lipofuscin deposition occurs with increasing age. It is possible that the increased vacuolation of lipofuscin occurring with ageing (Hasan and Glees, 1973a) may have been interpreted as a decrease in the quantity of lipofuscin occurring with time.
2.5 Biochemical Changes

2.5.1 Brain Lipids

"There is no other organ in the body that contains such a high proportion of lipids as the brain"
- Adams (1965)

Lipids are the major structural and functional constituents of any biological membrane. They serve as essential component of several crucial enzyme systems and as a fuel molecules, as highly concentrated energy stores (Lehninger, 1984). In the mammalian central nervous system, lipids comprise over half of the total dry weight (Brante, 1949; Balakrishnan et al. 1961; Ordy and Kaack, 1975; Suzuki, 1981). Almost all of the lipids in the CNS are found in membranes of cells. Different types of membranes accumulate different types of lipids. These brain lipids are constantly being synthesized replacing other molecules in the membranes (Horrocks et al, 1975). Neural membranes contain three major categories of lipids: cholesterol, sphingolipids and glycerophospholipids. These lipids are highly enriched in the CNS of vertebrates. Other lipids, such as triglycerides, free fatty acids and cholesterol esters, which are abundant in systemic organs, are minor constituents of the CNS (Suzuki, 1981). However, some of these lipids are metabolically very active. There is increasing evidence that lipid molecules play important functional roles within the membranes. Some of the postulated physiological functions of membrane lipids include the site for the cell-to-cell recognition process, specific cell surface antigens and specific receptors for toxins or other physiological compounds.
Brain contains a unique structure, the myelin sheath, which has the highest lipid concentration of any normal tissue or subcellular components, except adipose tissue. The range of values for myelin total lipid is 60-80% of dry weight (White et al., 1978). Myelin is present in all parts of the CNS, but is more concentrated in areas composed mainly of fibre tracts, such as the white matter of brain and spinal cord. Mammalian brain white matter contains about 50% myelin on a dry weight basis. Even in the whole brain of an adult rat, myelin is about 25% of dry weight and accounts for more than 40% of the brain lipid (Norton and Poduslo, 1973), because the lipid compositions of gray and white matter differ both in total concentration and in the distribution of individual lipids. Early analysis of white matter revealed that cholesterol, sphingomyelin and cerebroside were present in larger amounts than in the gray matter (Johnson et al., 1948). The complete lipid analysis of myelin from different species has been reported by various workers (Autilio et al., 1964; Eichberg et al., 1964; O'Brien, 1965). Substantial changes in lipid composition take place during active myelination. In active myelination, the brain loses water, predominantly in white matter. The lipid content increases rapidly and the differences between gray and white matter become more apparent (Wells and Dittmer, 1967; Norton and Poduslo, 1973). The rapidly increased myelin content is closely related with increase in brain weight (Smith et al., 1983).

It is well recognized that the brain undergoes distinct changes in lipid composition during growth and development that reflect the structural differentiation of the nervous system. As a result specific
patterns are formed during development of specific brain regions and subcellular membranes, particularly the myelin. Although a voluminous amount of information has already appeared to document these changes during development, little detailed information is available on lipid changes and ageing.

The studies by Rouser and Yamamoto (1968, 1969) on male human brain still remain one of the most comprehensive studies regarding quantitative changes in all the major lipid classes. They showed that lipid composition changes continuously throughout development and ageing, with individual lipid classes having different rates of change at different ages. Whole brain total lipid increases rapidly up to about 2 years of age, continues to increase less rapidly to a peak at 30-40 years, and then declines at a steady rate after the age of 50 (Rouser et al, 1972). The changes in the individual lipid classes have been summarized by Horrocks et al (1975, 1981). The greatest losses (12-19%) in lipids between 40 and 70 years of age found in cholesterol, cerebrosides, cerebroside sulphates, ethanolamine, plasmogens and sphingomyelins. All of these lipids are relatively enriched in myelin. These data are supported by Berlet and Volk (1980), who quantified myelin from white matter of human brain between the age of 17 and 89 years and found a significant decrease in myelin content in the aged brains.

Lipid changes in rodent brain differ from those in human brain. Increase in phospholipid, cholesterol and cerebroside content of aging brain of mouse, Peromyscus leucopus (long-lived field mouse) and rat have been reported by Sun and Samorajski (1972), Horrocks et al (1981)
and Bonetti et al (1983), respectively. These increases correlate well with reported increases in myelin content of aging rodent brains (Rawline and Smith, 1971; Norton and Poduslo, 1973; Malone and Szoke, 1982). These increases also correlate with specific increases of 60-80% in myelin galactolipids and cholesterol in mouse brain (Sun and Samorajski, 1972).

Studies from this laboratory have shown that chemical stress perturbs the levels of total lipids in the rat CNS (Tayyaba and Hasan, 1980; Islam et al, 1980; Hasan and Khan, 1985; Vadhva and Hasan, 1986 and Naqvi et al, 1988).

Little information is available on age-related changes with environmental stress until now. Because there is no report available on changes in various regions of brain and spinal cord during senescence, it would be of great interest to estimate the levels of total lipids quantitatively in the various regions of CNS of aged rats following restraint stress.

2.5.2 Phospholipids

Phospholipids are the major class of membrane lipids. Glycero-phospholipids are the major component of brain lipids. Phospholipids constitute approximately one fourth (20-25%) of the dry weight of brain (Holkin, 1969; White, 1973). Total amounts of phospholipids are higher in white matter than in gray matter, and the reported amounts range from 3.1-4.6g/100g fresh tissue for gray matter and 6.2-9.3g/100g for white matter (Yasuda, 1937; Randall, 1938; Brante, 1949; Johnson, 1949;
Suzuki, 1981). Large amounts of phospholipids in white matter evidently reflect their occurrence as a component of the molecular structure of myelin sheaths, 40-45% phospholipids present in the myelin sheath (Fumagelli and Paoletti, 1963). The importance of phosphorous containing lipids of the CNS presumably depends upon their role as membrane constituents. They not only constitute the backbone of biomembranes, but also provide the membrane with a suitable environment, fluidity and ion permeability (Farooqui and Horrocks, 1985; Nicotera et al, 1989). The phospholipid bilayer is penetrated to varying degrees by receptors, enzymes and ion channels, which differentially protrude through the membrane or are localized predominantly on the intracellular or extracellular membrane surface. One of the most important functions of the biomembrane is the regulation of ion homeostasis. The neural membranes are highly interactive and dynamic and therefore the interaction of ligand with receptor markedly affects neural membrane phospholipid metabolism (Loffelholz, 1989; Nahorski et al, 1986; Porcellati, 1983. This in turn regulates the microenvironment for the activities of the membrane-bound enzymes and ion channels (Farooqui and Horrocks, 1985). Porcellati (1983) and Procellati and his co-workers (1983) reported that different phospholipids turnover at different rates with respect to their structure and localization in various cells and membranes. Newly synthesized phospholipids are transported to membrane structure by phospholipid exchange and transfer proteins (Brammer, 1983; Demel et al, 1984) which are found in cytosol. The distribution of phospholipids in a biologic membrane is thus regulated not only by
the activities of enzymes involved in their metabolism but also by the transport and incorporation processes into the membrane. Many investigators such as Porcellati et al (1970a, b); Ansell and Spanner (1971) and Porcellati et al (1971) have concentrated on the regulatory mechanisms of phospholipid biosynthesis in the brain, and of the alternative metabolic pathways involved, should be of interest, because it is highly probable that the metabolism of phospholipids in brain is somewhat different from that in other tissues.

Phospholipids are of particular interest in ageing studies because they are an integral part of the membrane lipid bilayer and changes in composition may result in alteration of the membrane protein activities. More age-related differences have been found in acyl group compositions of glycolipids and phospholipids (Rouser and Yamamoto, 1969). The most dramatic differences are seen during early development. The important changes in the phosphoglycerides follow a general pattern of decreased properties of saturated and polyunsaturated acyl groups during development (Sun and Sun, 1972). Phospholipids were decreased with age in human brain (Rouser and Yamamoto, 1968). Phospholipids concentration increase by 30 µ moles/100g fresh weight during 23rd year, but decreases by 36 µ moles/100g fresh weight during 24th year. The annual decrease then becomes smaller during successive succeeding years (Svennerholm, 1968).

Myelin lipid, at the subcellular level in ageing brain has received greatest attention because this membrane is relatively early to purify
and pathological loss of myelin may be associated with loss of neuronal functions. Myelin phospholipids have been examined in some detail in ageing brain of mouse (Sun and Samorajski, 1972), rat (Rawline and Smith, 1971; Norton and Poduslo, 1973; Malone and Szoke, 1982; Smith 1973) and humans (Horrocks et al, 1981). These changes were observed in isolated myelin fractions and therefore do not merely reflect an increase in the proportion of myelin, which is relatively rich in monounsaturated acyl groups, relatively poor in saturated and (n-3) polyunsaturated acyl groups (Sun and Horrocks, 1970; Sun and Sun, 1972; Sun and Samorajski, 1972; Horrocks, 1973).

Recently, Gupta and Hasan (1988) from this laboratory have reported the changes in the cerebrum, cerebellum, brain stem and spinal cord phospholipids following restraint stress. To our knowledge, to date, only few attempts have been made to study the effects of stress on the phospholipid levels.

Neurobiochemical studies of regional phospholipid changes of rat CNS following 24 hours restraint stress have not been reported in the literature and present investigation provides necessary information on the effects of restraint stress on aged brain phospholipids.

2.5.3 Cholesterol

Cholesterol is a major sterol, present in significant amount in the central nervous system (Ansell and Hawthorne, 1964). It is also an important component of biological membranes. Membranes are generally thought to consist of phospholipid bilayers into which membrane proteins are
embedded, yet cholesterol molecules are present in most animal structures. Due to its amphipathic nature bearing an OH-group and a hydrocarbon skeleton with rigid rings and a branched chain of eight carbons, cholesterol is perfectly suited to mesh with lipid bilayers (Brenner, 1990).

Cholesterol accounts for about 10% of dry weight of the brain in contrast to less than 1% found in most other organs. The constancy of the amount of cholesterol in the brain suggests that the sterol is metabolically stable (Waelsch et al, 1940). Unesterified cholesterol has been suggested as a lipid characteristic of myelin sheath, as it occurs in white matter in a higher concentration than in gray matter (Johnson, 1949; Brante, 1949). About 25% of cholesterol is present in myelin lipid by weight (Soto et al, 1966) and approximately 70% of total brain cholesterol is present in myelin (Laatsch et al, 1962). Cholesterol is thought to act as a convenor in the absorption of fats. Bloor (1943) reported a parallelism between cholesterol content of blood and the fatty acids. Due to the abundance of cholesterol in nervous tissues and its variation in mental diseases, it may function as an insulating medium for myelin sheaths. Sterols are thought to have a role in maintaining the balance between the cell permeability and the membrane equilibrium of living cells. Brain microsomes are the side of cholesterol biosynthesis (Paoletti, 1971). Biosynthesis of cholesterol in brain is most rapid during active myelination, but adult brain retains the capacity to synthesize cholesterol when precursors, such as acetate or mevalonate are available.

Fetal or neonatal brain prior to myelination contains relatively little cholesterol. Kritchevsky and Holmes (1962) found varying amounts
of the sterol in the newborn rat brain. In rat brain, total levels of sterol ester increase from birth to 40 days (Eto and Suzuki, 1972). A decline is also noted in human and guinea pig brain cholesterol (62.3%) and desmosterol (31.1%) were the major sterol and small to trace amounts of other sterols were also detected (Ramsey and Nicholas, 1972) relative to total phospholipid and glycolipid, in which changes in whole brain and myelin are compared. Increase in cholesterol content of ageing brain of mouse and rat have been reported by Sun and Samorajski (1972), Horrocks et al (1981), and Bonetti et al (1983), respectively. These increases correlate with specific increases of 60-80% in myelin galactolipids and cholesterol in mouse brain (Sun and Samorajski, 1972).

Earlier findings from this laboratory have shown the alterations in the levels of cholesterol in discrete brain areas following chemical stress (Tayyaba and Hasan, 1980; Hasan and Khan, 1985; Vadhva and Hasan, 1986; Naqvi et al, 1988). Little information is available on the effects of restraint stress on ageing (Gupta and Hasan, 1988). Therefore, it would be of interest to estimate the levels of cholesterol quantitatively after the restraint stress in various age groups of rats.

2.5.4 Triglycerides

Triglycerides consists of glycerol esterified with 1,2 or 3 fatty acids. They are synthesized in the liver and in most other tissues from alpha-glycerophosphate and the coenzyme-A derivative of the long chain fatty acids. Triglycerides are the most abundant form of the fat in the mammalian body. The mass of the fat stored in adipose tissue depots
contains more than 90% of triglycerides. This mass of fat makes up 10-35% of body weight (Weidman and Schonfeld, 1980). Triglycerides are present in the plasma in association with other lipids and with proteins in lipoprotein complexes. These can best be considered in the present context as providing a mechanism for the transport of a water-soluble triglyceride in an aqueous medium (White et al., 1978; Smith et al., 1983). Two lipoprotein classes - the chylomicrons and the very low density lipoproteins (VLDL) carry most of the plasma triglycerides. The concentration of triglycerides in the plasma at any time reflects a balance between rates of entry and removal of triglyceride fatty acids from the circulation.

The brain is unique in its lack of certain lipids that are abundantly present in other organs of the body. However, triglycerides constitute at the most only a few percent of the total lipid of the brain lipids, some are probably contributed by blood and blood vessels, rather than by neural tissues (Suzuki, 1981; Cook, 1981; Horrocks and Harder, 1983).

2.5.5 Gangliosides

Gangliosides are the sialic acid containing glycosphingolipids, which are highly enriched in the CNS of vertebrates, including man (Wiegandt, 1967; Ledeen, 1978; Svennerholm, 1980). Sialic acid is the generic name for N-acylneuraminic acid, and the acyl group of sialic acid in the human brain is always the acetyl form (Suzuki, 1981). N-acetytnleuraminic acid is commonly abbreviated as Nac. In the gray matter 6%
of the total lipids are gangliosides (Lehninger, 1984). Also gray matter contains many times higher ganglioside concentration, compared to white matter (Wolfe, 1972). In the brain, at the cellular level, gangliosides seem to be more highly concentrated in neurons (Yu and Iqbal, 1979) than the glial cells (Roberts et al, 1975) and myelin (Ledeen, 1978). Many investigators have thought that each component of neural tissue would possess a specific set of gangliosides and that a definite set of gangliosides might be required for particular membrane functions. Gangliosides are localized in two fraction of the brain. Major gangliosides of the brain are GM₁, GM₂, GM₃, GD₁a, GD₁b and GT. Several other minor gangliosides have been identified in the nervous system including GD₃, GD₂ and sialylagalactosylceramide. A minor amount of ganglioside appears to serve as a structural role in the myelin matrix (Suzuki, 1981). The bulk of the ganglioside in brains is found in the outer membrane of the nerve endings. Wiegandt (1967) has suggested that they are involved in nerve impulse conduction. It has been demonstrated that the distribution of gangliosides resembles that of ψ-aminobutyric acid (Lowden and Wolfe, 1964). Deul and his coworkers (1968) have indicated that the binding of serotonin to synaptic vesicles may be mediated by gangliosides. Irwin (1969) and Irwin and Samson (1971) have also indicated that certain types of behavioural stimulation seem to be accompanied by alteration of ganglioside metabolism compared to corresponding control animals. Gangliosides have also been shown to modulate the phosphorylation system (Agnati, 1985 Bremer et al, 1986). Gangliosides in brain play an active role rather than a passive function.
Gangliosides, during development undergo characteristic changes in content and composition (Rosner, 1977; Engel et al, 1979; Yusuf and Dickerson, 1980) and have, therefore, been suggested to be instructional molecules for brain maturation. In particular, they appear to be functionally involved in the control of axonal (Willinger and Schachner, 1980) and dendritic (Irwin and Irwin, 1979) out growth, synaptogenesis (Engel et al, 1979) and the establishment of cell contact (Yogeeswaran and Hakomori, 1975). Moreover, in adult nervous system, the individual gangliosides have been suggested to play a role as membrane-bound receptors or co-receptors for toxins, drugs viruses, hormones, transmitters etc. (Svennerholm, 1980). In many ways ageing of the brain can be seen as the reversal of these events, with neurons and synapses being lost (Brody and Vijayashankar, 1977; Bondareff, 1980) dendrites deteriorating (Scheibel and Scheibel, 1975; 1977 and myelin sheath of neurons degenerating progressively (Berlih and Wallace, 1976). This leads to the assumption that brain gangliosides also change with ageing, which has in fact been shown for the rat (Rahmann 1980; Horrocks et al., 1981) and humans (Rouser and Yamamoto, 1968, 1969; Cherayil, 1969; Fredman et al, 1980; Seglar-Sthal, 1983). The total ganglioside content of human brain appears to increase upto about 6 to 8 months constantly and then declines (Rouser and Yamamoto, 1968). In human brain, the concentration of gangliosides-bound sialic acid drops by 47% from age 25 to 48 years, plateaus until the mid 70s and then begins to drop again to a low level of 400 ug/g at age 85 (Seglar-Sthal, 1983). Fredman et al, (1980) reported than the brain of a 65 years old contained more tetra and trisialogangliosides by less disialogangliosides than the brain of 3 months old.
A similar rise upto about 20 days and then a fall through at least 50 days of age is apparent for rat brain (Suzuki, 1967). Merat and Dickerson (1973) reported that in the fore-brain of rat the proportion of disialogangliosides decreased and monosialogangliosides increased (about 40%) between 17 and 27 months. The sialic acid level of rodents declines only slightly during senescence (Rahmann, 1980). The decline of ganglioside concentration during ageing is probably related to degradation of the ganglioside rich components such as neuronal membrane. However, the cause-effect relationship has not been delineated (Seglar-Sthal, 1983).

To date, little information is available on the ganglioside concentration in rat brain following restraint stress (Gupta and Hasan, 1988). Therefore, the present study deals with the effect of restraint stress on gangliosides concentration in different age groups of the rat brain.

2.5.6 Lipid Peroxidation and their Products

"All the diseases are the result of collection of waste materials, the latter being initiated by many causes to produce symptoms; wastes collect due to incorrect dieting and living."

- Atharva Ved.

The lipids within the membranes of cells from higher organisms contain large number of polyunsaturated fatty acid sidechains. Such fatty acids are prone to undergo a process known as "lipid peroxidation", which involves the generation of carbon radicals followed by production
of peroxide radicals (Sohal, 1981). Lipid peroxidation has been identified as a basic deteriorative reaction in the cellular mechanism of ageing process, in cells damaged by environmental pollutants and in variety of pathological conditions (Tappel, 1973). Biomembranes and subcellular organelles are the major sites of lipid peroxidation (Tappel, 1970). Quantitative studies of enzymatic inactivation by lipid peroxidation have shown that sulfhydryl enzymes are most susceptible to inactivation (Chio and Tappel, 1969; Green et al., 1971; Rubstov et al., 1984; Leelarc et al., 1987; Numan et al., 1990). The brain which is rich in polyunsaturated fatty acids and oxygen supply and has low activities of antioxidant defense enzymes becomes a vulnerable organ for such peroxidative attack.

Lipid peroxidation is an autocatalytic free radical process (Pryor, 1987). Free radicals are short lived, highly reactive chemical species involved in a variety of functions, namely, oxidation of unsaturated fatty acids in cell membranes (lipid peroxidation), damage of DNA, modulation of nucleotide cyclase activities and synthesis of prostaglandins and lipo-peroxides. These free radicals are usually produced in biological system during anti-microbial defense, through the action of mixed function monoxygenases, by various oxidative enzymes, such as xanthine oxidase and by auto-oxidation mediated by heavy metals and quinones (Proctor and Reynolds, 1984; Richter, 1988; Simic, et al., 1989). The H$_2$O$_2$ and other reactive O$_2$ species, if not scavenged efficiently, are known to give rise to potentially toxic intermediates, namely, hydroxy radical (°OH) and singlet (°2). These oxidants, in the presence of metal ions, result in the formation of lipid peroxides (Lai and Piette, 1978; Lai et al., 1979a).
Initiation of lipid peroxidation in a membrane or free fatty acid is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom. Since a hydrogen atom has only one electron, this leaves behind an unpaired electron on the carbon atom. The carbon radical in a polyunsaturated fatty acid tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which rapidly reacts with $O_2$ to give hydroperoxy radical. Hydroperoxy radicals abstract hydrogen atoms from other lipid molecules and so continue the chain reaction of lipid peroxidation. The hydroperoxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxide (Barber and Bernheim, 1967). The classically accepted mechanism of free radical lipid peroxidation is outlined below.

$[\text{LH} = \text{fatty acids}; \ \text{LOOH} = \text{lipid hydroperoxides}; \ \text{L}^\cdot = \text{lipid alkyl radical}; \ \text{LOO}^\cdot = \text{lipid peroxy radicals}]$ (Aust and Svingen, 1982).

**Initiation**

$$\text{LH} + O_2 \rightarrow \text{Free radicals, L}^\cdot$$ (i)

$$\text{LOOH} \rightarrow \text{Free radicals, LOO}^\cdot$$ (ii)

**Propagation**

$$\text{L}^\cdot + O_2 \rightarrow \text{LOO}^\cdot$$

$$\text{LOO}^\cdot + \text{LH} \rightarrow \text{LOOH} + \text{L}^\cdot$$

**Termination**

$$\text{LOO}^\cdot + \text{LOO}^\cdot \rightarrow \text{LOOL} + O_2$$

$$\text{LOO}^\cdot + \text{L}^\cdot \rightarrow \text{LOOL}$$

$$\text{L}^\cdot + \text{L}^\cdot \rightarrow \text{LL}$$
A scheme for free radical initiation of lipid peroxidation is shown in Fig. 2.6.

Lipid peroxidation and its products are toxic to various cells. Lipid hydroperoxides decompose to produce aldehydes (e.g. malondialdehyde) and other products including gaseous hydrocarbons such as ethane and pentane (Pryor, 1978; Konze and Elstner, 1978; Cohen, 1979). Their decomposition is catalyzed by transition metal ions and by haem compounds (O'Brien, 1969; Graziano, 1976; Kohn and Kessel, 1979). Lipid hydroperoxides and some of their degradation products are highly cytotoxic: they cause extensive damage to enzymes and to membranes, producing a decrease in electrical integrity (Gardner, 1979; Pauls and Thompson, 1980; Sohal, 1988; Watanabe et al., 1990). DNA can be damaged and so lipid peroxidation can have a mutagenic effect (Saul et al., 1987; Wilson, 1987). Further, there is some evidence that malondialdehyde is a mutagen (Summerfield and Tappel, 1984; Black et al., 1985). Disruption of lysosomal membranes by lipid peroxidation can spill hydrolytic enzymes into the rest of the cell and thus potentiate the damage (Fig. 2.7).

Age-pigment is an end product of lipid peroxidation. The accumulation of fluorescent age-pigment or lipofuscin is a frequently observed age-associated cellular alteration in a variety of post-mitotic cells of many species (Wolmen, 1975). Free radical intermediates of lipid peroxidation react with protein causing denaturation and polymerization (Balin, 1982). The co-polymerization of lipids with proteins, peptides or other
HYDROGEN ABSTRACTION - BY A PREVIOUSLY FORMED PEROXIDE RADICAL OR BY A SPECIES SUCH AS OH+ GENERATED FROM O₂

REARRANGEMENT

CONJUGATED DIENE (CAN BE DETECTED BY ABSORBANCE AT 233 nm.)

PEROXIDE RADICAL: ABSTRACTS H+ FROM ANOTHER CHAIN SO CAUSING A CHAIN REACTION.

HYDROPEROXIDE

FIG. 2.6. MECHANISM OF PEROXIDATION OF POLYUNSATURATED FATTY ACIDS.
Fig. 27. MECHANISMS OF FREE RADICAL CHAIN REACTIONS LEADING TO FORMATION OF UNSATURATED FATTY ACID HYDROPEROXIDES. HOMOLYTIC DECOMPOSITION OF HYDROPEROXIDE LEADS TO CHAIN BRANCHING REACTIONS OR AUTOCATALYSIS.
compounds results in the formation of cellular metabolites in the cells (Hasan and Glees, 1972a; Patro et al., 1988). The involvement of free radicals in the lipofuscin formation has been demonstrated in human glial cells and rat heart monocytes by Thaw et al. (1984) and Sohal et al. (1989a), respectively.

Membrane damage during ageing is one of the major destructive process of lipid peroxidation (Tappel, 1973; Chasovnikova et al, 1990). Watanabe et al (1990) ascertained that alterations in biophysical properties of membranes caused by lipid peroxides play an important role in cell injury. High levels of lipid peroxides have been related to a number of degenerative diseases (Pryor, 1987). Fujiwara et al, (1990) suggested that the decreased antioxidant injury in hypoxemic patients enhances lipid peroxidation in erythrocytes. Old age is associated with the higher lipid peroxidation capacity and the decreased inducibility of defensive enzymes (Imre and Juhasz, 1988). Nomura et al (1989) found higher levels of MDA in brain and liver of senescence-accelerated mouse which was accompanied by a loss of superoxide-dismutase (SOD) activity. Lemeshko et al (1987) showed that the intensity of peroxidation of bio-membranes lipids gets lowered in liver and brain of ageing rat. They associated it with an increase in antioxidant enzymes. The capacity of MDA oxidation is lost progressively with age and the extent of loss is modulated by food restriction (Wadhwa et al, 1986; Kim and Yu, 1989; Govinda et al, 1990). Age-associated increase in lipid peroxidation was also observed in rat liver microsomes (Sagai and Ichinose, 1980), human serum (Hagihara et al, 1984) and human red cells (Nakai et al,
Videla et al (1987) reported that total liver thiobarbituric acid reactants (TBARS) showed an increase at 39 weeks of age as compared to the young (3 weeks), followed by a diminution in 53 weeks' old groups. Age-related increase in lipofuscin has also been observed in Caenorhabditis elegans (Klass, 1977), Drosophila melanogaster (Miquel et al, 1974; Siedahl and Tappel, 1974) and Musca domestica (Sohal and Sharma, 1972; Donato and Sohal, 1978; Donato et al, 1979a, b). Muscari et al (1990) found increased lipofuscin content with age in glutamate and succinate induced respiration in male wister rats. The fluorescent intensity and the optical density (OD) of lipofuscin granules of retinal pigment epithelium were also found increased with age by Boulton et al (1990).

2.5.7 Antioxidant Defence Systems

Free radical reactions are ubiquitous in living beings because of the high chemical reactivity of the intermediates. Various pathways are known by which free radicals can mediate cellular toxicity. The action of free radicals on biological system has the potential for disturbing the balance of pro-oxidants and antioxidants. An alteration in this balance in the favour of pro-oxidant is known as oxidative stress (Sies, 1985). To control and reduce the free radical induced cellular damage, the organism has a compensatory mechanism, which comprises the most important variables in controlling or preventing free radical reactions. These defences include some naturally occurring antioxidants as well as exogenous agents that have been proved useful. Some of these are water soluble or confined exclusively to non-polar environment such
as ascorbic acid and tocopherols, respectively. The other anti-oxidants that have received maximum attention in biological systems, include selenium and the thiol containing compounds like glutathione and the enzymes of glutathione cycle (Flohe et al, 1976; Kosower and Kosower, 1978) (Fig. 2.8). Antioxidants are divided into two major classes:

(i) Preventive antioxidants: These antioxidants interfere with initiation of the free radical chain reaction, e.g., catalase and other peroxides and the chelators of the metal ions.

(ii) Chain breaking antioxidants: They interfere with chain propagation. They comprise superoxide dismutase, which acts in the aqueous phase and reduces the superoxide anion; similarly uric acid and vitamin E act in the lipid phase to reduce the lipid peroxy radicals (Chance et al, 1979; Halliwell and Gutteridge, 1985).

2.5.7.1 Sulfhydryl groups

Sulfhydryl (-SH) group is also known as thiol group. It plays a key role in many important enzymes by acting as active enzymatic sites (Hoch and Vallee, 1959). In principle, any enzyme bearing an accessible thiol, essential for activity, is capable of forming protein mixed disulfides or intramolecular disulfides by reacting with small disulfides. Formation of mixed disulfides or intramolecular disulfides can increase or decrease catalytic activity and examples of both are known. Furthermore, the extent of enzyme-S-thiolation would depend on the thiol-disulfide redox potential as well as the nature of the small disulfide
FIG. 2.8. PATHWAYS OF ENZYMIC DETOXIFICATION OF SUPEROXIDE AND ITS REACTION PRODUCTS.
and the microenvironment around the accessible protein thiol. These parameters are at least potentially capable of conforming the specificity required for a biological control mechanism through signals transmitted by changes in the thiol-disulfide redox potential as a function of different metabolic states.

Sulfhydryl groups derived from the side chain of cysteine residues, occur in a number of enzymes. Sulfhydryl (-SH) group and disulfide (-SS) bond of cysteine are highly reactive and apparently involved in the maintenance of the conformation and biological activity of certain proteins. As the receptors are protein in nature, the reagents which modify -SH groups may influence the interaction of neurotransmitters with their recognition sites (Sobrino and Del Castillo, 1972).

Sulfhydryl groups play an important role in GST induced detoxification against electrophilic xenobiotics and toxicants by conjugating with such compounds and thus neutralizing their electrophilic sites (Habig et al, 1974).

Glutathione has been considered to function as biological antioxidant. It plays a pivotal role in the destruction of free radicals as well as inorganic and organic peroxides (Sohal et al, 1984). GSH is a naturally occurring and widely distributed tripeptide. It consists of glycine, cysteine and glutamic acid moieties (Allen and Balin, 1989). It is the major non-protein thiol compound present in cells in concentrations which range between 0.1 and 10 mM (Kosower, 1976a). It is synthesized intracellularly by the consecutive actions of -glutamyl cysteine synthase and GSH
synthase. Its concentration is dependent on metabolic rate and the level of oxidative stress (Allen et al, 1985a). It has been implicated in a wide variety of biological functions, such as the maintenance of cell membranes, destruction of metabolic peroxides and free radicals, detoxification of foreign compounds, removal of $H_2O_2$, maintenance of thiol groups of enzymes and proteins, control of redox status, disulfide exchange reactions, transport of amino acids and peptides across membranes (Hazelton and Lang, 1980; Meister and Anderson, 1983; Ziegler, 1985).

The concentration of GSH of tissue is inversely proportion to ageing. Younes and Siegers (1980) and Katoh et al (1989) observed an enhanced level of lipid peroxides associated with the GSH depletion. The role of GSH in peroxidation is evidenced by the inhibition of oxidative stress induced by different compounds such as ascorbate, NADPH-BrCCl$_3$ and NADPH-Fe$^{2+}$ (Wefers and Sies, 1988; Tampo and Yanaha, 1990). The GSH was observed to protect rats from toxic 0 species engendered by hyperoxia (Van et al, 1985). White et al (1988) observed that GSH redox cycle increases survival and detoxification of $H_2O_2$ in hypoxia pre-exposed rats and contributes to tolerance to hyperoxia. Gupta et al (1986) observed a significant increase in GSH level with antioxidant in mice. Rotruck et al (1972) reported that Se-glucose-GSH system plays a dual role in the preservation of the integrity of the cells membrane and of haemoglobin against haemolysis and oxidative damage.

Tappel (1970) has suggested that deficiency of total and free sulfhydryl groups may lead to deficient degradation of lipid peroxides to hydroxy acids, causing accumulation of peroxides in various regions of the brain.
The concentration of oxidized glutathione or glutathione disulfide (GSSG) reported for various tissues range between 4 and 50 μM (Tietze, 1969). According to Kosower and Kosower (1974a) a slight increase in the concentration of GSSG even in the presence of a large excess of GSH, has effects of potential physiological importance. One potent physiological function of the activity of GSSG in inhibiting protein synthesis might be as a control mechanism. If the concentration of GSSG within the cell rises above a certain level, initiation factors are converted into an inactive form and the total rate of protein synthesis decreases. Alterations in the GSH/GSSG ratio may also be related to the enhanced rate of protein synthesis (Zehavi-Willner et al, 1971).

2.5.7.2 Superoxide dismutase

All aerobic organisms utilize O2 and must have some mechanism by which they can minimize O2 toxicity. One mechanism is the production of superoxide radical and its dismutation reaction, catalyzed by the enzyme superoxide dismutase (Harman, 1956; 1971). The superoxide anion is a free radical formed by one electron transfer to oxygen.

\[
O_2 + e^- \rightarrow O_2^*^{-}
\]

Superoxide dismutase (SOD) catalyzes the dismutation between two moles of superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (hydrogen peroxide) (Klug, et al, 1972).
\[
O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2
\]

This is analogous to the dismutation of hydrogen peroxide to oxygen and water catalyzed by catalase. Ordinarily, electrostatic repulsion between two molecules of superoxide anion limit their approach to one another; SOD overcomes the barrier and greatly increases the dismutation rate (Fridovich, 1976; 1978).

SOD has been demonstrated in a variety of tissues and cell types and appears to protect against the toxic effects of the \(O_2^-\) free radical and thus provides a mechanism whereby an organism can avoid possible deleterious effects of this radical or other free radicals which might be produced by its further reaction with cellular components (Fridovich, 1975; McCord et al, 1971). Superoxide arises naturally in some enzymatic reactions (Fridovich, 1978) such as xanthine oxidase, dihydro-orotic acid oxidase, aldehyde oxidase, tryptophan dioxygenase or during auto-oxidation of tissue constituents such as reduced flavins or ascorbate or more dramatically during the rapid spontaneous auto-oxidation of certain neuronal toxins such as 6-hydroxydopamine or 6-aminodopamine (Cohen and Heikilla, 1974). Superoxide radical at neutral pH can act either as a weak oxidizing agent, e.g. with catecholamines, or as a strong reducing agent, e.g. with cytochrome C or nitroblue tetrazolium.

Several forms of SOD have been identified since the enzyme was first discovered in 1969 by McCord and Fridovich. They identified the enzymatic activity associated with erythrocuprein, a copper-zinc protein of erythrocytes. The copper is associated with enzymatic activity,
whereas the zinc is structural. Similarly, SOD activity is associated with a family of copper-zinc proteins, cerebrocuprein in brain (Fried, 1979) and hepatocuprein of liver. In mammalian tissues, a second form exists in which manganese is the prosthetic group (Fridovich, 1976). In rats and mice the Mn SOD is localized to mitochondria, whereas the Cu-Zu SOD is cytoplasmic. However, this distribution does not hold in other species.

Fried and Mandel (1975) indicated that very high levels of activity are present in liver, while the adrenals, kidney and red blood cells have intermediate activity and lower activities were found in most other tissues including brain. Regional distribution studies in the rat by Thomas and his coworkers (1976) showed a relatively homogeneous distribution in brain about a two-fold range from the highest area (medulla oblongata) to the lowest area (cortex). Subcellular distribution studies in the rat (Thomas et al, 1976) showed the highest level in the cytoplasm while myelin has very low levels.

Peroxidative damage has been implicated as one of the principal causes of age-related damage to cells (Barber and Bernheim, 1967; Hougarrd, 1968). Such damage is at least partially associated with the free radicals. The reduction in SOD activity as a function of age could result in an impaired protection against the toxic effects of $O_2^{-*}$ and thus might lead to severe cellular damage (Kellogg and Fridovich, 1976; Massie et al, 1979; Vanella et al, 1982, 1989; Benzi et al, 1988a; Tayarami et al, 1989).
Oxidative and chemical stress (Shukla et al., 1987) inhibited SOD activity in various regions of the brain in growing rats. They suggested that stress directly and indirectly through inhibition of SOD increases lipid peroxidation in cell membranes and this produces damage to the associated physiological function, leading to CNS dysfunction.

2.5.7.3 Catalase

Catalase is another scavenging enzyme which catalyzes the breakdown of hydrogen peroxide to water and oxygen (Scott and Harrington, 1990).

\[ 2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \]

It is a tetrahemin enzyme. Recent studies by Kirkman et al. (1987) have revealed that each tetrameric molecule of human and bovine catalase has four molecules of tightly bound NADPH. In CNS, \( H_2O_2 \), a known cytotoxin, is produced during amine metabolism (Tipton, 1968). Catalase reduces \( H_2O_2 \) and thus serves a protective role in brain metabolism. CNS catalase is largely associated with small subcellular particles termed as "microperoxisomes" (Hruban et al., 1972; Novikoff et al., 1973; McKenna et al., 1976). It exerts various physiological functions. A protective role for catalase in brain is the removal of \( H_2O_2 \) which can induce damage to tissue constituents by oxidizing enzymes or membrane sulfhydryl groups or by initiating lipid peroxidation. This has led Gaetani et al. (1989) to demonstrate that catalase is equally involved in the removal of
H₂O₂ as is GSHPx. Furthermore, at higher concentration of H₂O₂, the action of catalase becomes increasingly important than GSHPx (Cohen and Hochstein, 1963). It has been tested that catalase prevents ascorbate-induced somatic mutation (Rosin et al, 1980), free radical-induced aldehyde formation in DNA (Sinha and Patterson, 1983), lipid peroxidation (Koster and Slee, 1980 and DNA scissions caused by H₂O₂ (Chilou, 1983; Yonei et al, 1987). The increased H₂O₂ concentration and lipid peroxides levels are often associated with the decreased catalase activity (Jana and Choudhuri, 1982; Del Boccio et al, 1990; Vani et al, 1990). In many systems, catalase and SOD work together to eliminate the toxic precursors of free radicals (Fridovich, 1981). Many available reports indicate that SOD and catalase form a "defensive team" effective against endogeneously produced superoxide anion (O₂⁻) (Rowley and Halliwell, 1983; Potmesil et al, 1984; Ahmed et al, 1987).

Bhargava and Shanker (1981) suggested that the absence of catalase from brain makes it more vulnerable to the higher concentration of ascorbic acid and the lower concentration of GSH. Catalase inhibition by aminotriazole increased the endogeneous brain peroxidation in frog while survival of the animal was not affected (Barja De Quiroga et al, 1990). Exogeneously added catalase prevented the H₂O₂ induced cell lysis (Schraufstalter et al, 1985) and protected the mutant cells from growth inhibition (Egashira et al, 1989). Higuchi et al (1985) could not find any adaptive change in catalase activity in the exercise-trained skeletal muscle of rats while catalase activity was stimulated in response to peroxidative damaged caused during the course of cryogenic rabbit brain
edema (Averet et al, 1990). An adaptive increase catalase activity was also observed with the increased myocardial lipid peroxide level during adriamycin-induced cardiotoxicity (Tanaka and Takahashi, 1990). Guner et al (1985) observed decreased $\text{H}_2\text{O}_2$ detoxification by catalase in human brain tumoral tissue as compared to normal. Contrarily, the absence of catalase activity was reported in tumor cells by Muhammed and Kurup (1984).

Age-related changes in catalase activity have been observed in various organisms, e.g. *M. domestica* (Sohal et al, 1984, 1985a) and rats (Yam et al, 1978; Schisler and Singh, 1987). An increase of catalase activity occurs during growth and a decline towards senescence in *Drosophila* (Samis et al, 1981; Nickla et al, 1983). Age-related decline was also observed in rat lung by Yam et al (1978). Imre and Juhasz (1988) concluded that the declined adaptation capacity of ageing mice is associated with age-dependent diminution of catalase activity and found a decreased inducibility of higher catalase activity on $\text{H}_2\text{O}_2$ supplementation in adult and aged mice. Contrarily, Vanella et al (1987) found a significant increase in catalase activity during ageing in hippocampus and cerebral cortex in rat brain. However, no change in catalase activity was reported by Roy et al (1983) in rat brain, Yoshioka et al (1980) in rat lung. According to Ansari et al (1989) catalase activity declined with age in various regions of brain. The results of these studies depended largely on which organism was used, and which antioxidants were measured.

It is widely held that there is a decreased capacity to deal with stress with increasing age. Accumulation of oxidatively modified cellular
components suggests that aged organisms may be less capable of coping with oxidative stress.

2.5.7.4 Glutathione reductase (GSSG-reductase; GSSG-R or GR)

Glutathione reductase, a FAD dependent, heat labile enzyme catalyzes irreversible conversion of GSSG to GSH and accounts for very high GSH:GSSG ratio in the cells. The reaction takes place according to the following equation.

\[
\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GSSG-R}} 2\text{GSH} + \text{NADP}^+
\]

Glutathione reductase is reckoned to be as ubiquitous as glutathione and has been studied in various tissues (Ray and Prescott, 1975; Moron et al., 1979; Ormstad et al., 1979). This enzyme is found in the isolated from human platelets (Moroff and Kosow, 1978), leucocytes (Ogus and Tezcan, 1981) and erythrocytes (Chang et al., 1978; Krauth-Siegel et al., 1982). The primary and unambiguous role of glutathione reductase is of course, to regenerate reduced GSH that has been oxidized (i) non-specifically by oxygen radicals or peroxides, (ii) enzymatically through the GSH peroxidase reaction, (iii) spontaneously or enzymatically by means of thiol-disulfide exchange reactions or (iv) possibly by other redox reactions.

Glutathione reductase has been illustrated to be an inducible enzyme when rat liver cells were treated with various compounds which suggests that GSSG-R is of great importance to the production of cells against
toxic agents (Carlberg et al., 1981). The destruction of GSHPx, GSSG-R, SOD and catalase activities was found to be the underlying cause of free radical damage caused by reperfusion injury of rat kidney (Okajima, 1990). Benzi et al (1989) measured the activities of enzymes related to the anti-oxidant system in different regions of brain of ageing rats. In general, both SOD and GSSG-R tended to decline during the last half of life. Santa and Machado (1986) observed that GSSG-R activity was maximum at 25 days after birth in rats, afterwards the activity decreased continuously in adults but again increased during the ageing period, especially in female rats. Al-Turk et al. (1987) found that GSSG-R activity and GSH content of mice lymphocytes were higher in 2 months and 24 months old mice as compared to 9 months old. Stohs et al. (1984) reported that GSSG-R activity and GSH content were higher in erythrocytes from mature and middle aged human followed by a considerable decline contributing to senescence and increased susceptibility to carcinogenesis and drugs.

2.5.7.5 Glutathione peroxidase (GSHPx)

Glutathione peroxidase (glutathione : $\text{H}_2\text{O}_2$ oxireductase) was demonstrated by Mills (1957) to be a peroxidase in red blood cells and in a variety of tissues. Little and O'Brien (1968) Christophersen (1969a) demonstrated that the enzyme would detoxify lipid peroxides by converting the peroxides to their corresponding monohydroxy unsaturated fatty acids. The reduction takes place at the expense of donor substrate, GSH, which is hydrogen donor to reduce hydroperoxides to the corresponding alcohols.
$2\text{GSH} + \text{ROOH} \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{GSSG}$

$R$ may be $H$ or an organic residue. These acceptor substrates comprise a variety of biochemically important compounds, such as unsaturated lipids, steroids, nucleic acids (Flohe and Gunzler, 1974) and prostaglandins (Nugteren and Hazelhof, 1973). Thus, it plays a key role in modulating the GSH/GSSG ratio and indirectly affects the NADP/NADPH quotient of cell.

The enzyme occurs in two forms (i) selenium dependent GSHPx - it catalyzes the reduction of all hydroperoxides including $\text{H}_2\text{O}_2$, (ii) selenium independent GSHPx : it catalyzes the break-down of only organic hydroperoxides.

The presence of peroxidase in various tissues and the ability of the enzyme to metabolize peroxides of any structure at similar rates has led to the suggestion that glutathione peroxidase is the main product within the mammalian cell from peroxidative damage (Christophersen, 1969a, 1969b; Flohe and Zimmermann, 1970; Chow and Tappel, 1972; Chow et al, 1973). GSHPx has been shown to regulate multiple cellular functions, such as cell division (Kosower and Kosower, 1974a) pentose phosphate shunt (Eggleston and Krebs, 1974) and mitochondrial oxidation of 2-oxoacids (Sies and Moss, 1978). The role of the GSHPx in maintaining the integrity of the erythrocyte membrane has been extensively studied (Valentine and Tamaka, 1972; Beutler, 1972).
Owing to the high concentration of polyunsaturated fatty acids to peroxidative damage GSHPx could provide a mechanism to protect brain tissue against this type of damage. A limited number of studies have been performed with brain. A species comparison of levels of GSHPx in the cytosolic fraction of brain was reported by Demarchina et al (1974). The study was performed with unperfused brains may be subject to contamination by erythrocytes which contain much higher levels of enzyme. A subcellular distribution study carried out in perfused rats (to remove brain erythrocytes) by the same authors showed little or no enrichment when the whole homogenate was compared to mitochondrial membranes. The soluble protein fraction showed highest activity, whereas the microsomal fraction showed the lowest.

Su et al. (1979) reported an age-related increase of GSHPx in kidney of mouse while blood GSHPx showed an increase only in vitamin E supplemented animals. Other tissues, like lung, liver, uterus and spleen, did not show any age-related increase in the enzyme activity. Simonetti et al. (1990) found the GSHPx and catalase increased with age and attained their highest values by adulthood or senescence respectively in the subendocardial region of heart. Hazelton and Lang (1983) observed that GSHPx and SOD showed a lower constant specific activity during fetal development with a post-natal increase up to adult age of isolated rat hepatocytes. Hydrogen peroxides inhibited GSHPx activity (Kihlstrom et al, 1986; Ochi, 1990 b) while Lemeshko et al. (1985) reported that $H_2O_2$ cumene hydroperoxide and t-butyl hydroperoxide as substrates
increased GSHPx activity more than 1.5 fold over the period of 1-12 months and maintained high in 24 months old rats. Following vigorous exercise, plasma lipid peroxide concentration was increased and GSHPx activity significantly reduced.

Two potential development studies of GSHPx in rat the brain have been reported. Brannan et al (1981) used perfused rat brain and compared the developmental change of both GSH peroxides and GSSG reductase from birth to adulthood. Prohaska and Ganther (1976) noted a sharp fall in brain GSHPx during the first 2 weeks, followed by return to original birth levels by day 30.

2.5.7.6 Glutathione-S-transferase (GST)

Glutathione-S-transferase (GST; EC 2.5.1.18) is a non-selenium dependent glutathione peroxidase (Sies et al., 1979). GST was first identified in 1961 (Booth et al., 1961; Coombs and Stakelum, 1961). The enzyme was subsequently named glutathione-S-aryl transferase. Later on, several other GSTs were demonstrated depending upon the substrate specificity. Following types of GSTs have been described so far -

(i) Glutathione-S-alkyl transferase, catalyzing the conjugation of a variety of alkylhalides with glutathione (Johnson, 1966);
(ii) Glutathione-S-epoxide transferase, active towards the conjugation of active epoxides with glutathione (Boyland and Williams, 1965);
(iii) Glutathione-S-alkene transferase, catalyzing the conjugation of unsaturated compounds with glutathione.
These enzymes are ubiquitous in nature, and GST activity has been identified in man, non-human primates, rats, mouse, hamster, guinea pig, chicken, cow, sheep, trout and shark (Mannervik, 1985). The concentration of GST is, in general, high in mammals (upto 10% of cytosolic proteins in some organs), in other species (shark) the level of activity is quite low (Sugiyama et al., 1981). In addition, it is generally present in most mammalian organs.

The GSTs are a family of multifunctional proteins that function both as important enzymes of detoxification and intracellular binding proteins (Boyer, 1989). As enzymes, they catalyze the reaction between nucleophil reduced GSH and large number of electrophilic compounds such as polycyclic aromatic hydrocarbons, aromatic amines, azodyes, alkylating agents, carcinogens and neurotoxins (Boyland and Chasseaud, 1969; Habig et al., 1974; Jakoby, 1978; Chasseaud, 1979). They also bind a number of amphipathic compounds that they do not metabolize (non-substrate ligands) and have been suggested to act as intracellular transport proteins for compounds that have limited solubility in water (Levi et al., 1969). Additionally, a number of endogeneous compounds, including prostaglandins, leucotrienes, organic hydroperoxides (including lipid hydroperoxides and products of lipid peroxidation) and steroids act as substrate for GST (Mannervik, 1985; Jakoby, 1978; Chasseaud, 1979; Kaplowitz, 1980). However, it is important to note that hydrogen peroxide is not a substrate for GST (Lawrence and Burk, 1976).

Two types of products are produced by GST-catalyzed reactions (Douglas, 1987). In one type of reaction, a stable glutathione conjugate
is formed by the nucleophilic attack of GSH on an electrophilic center. These types of reactions occur with substrates such as epoxides (metabolites of benzo (a) pyrine and aflatoxin A) alkyl and amyl halides (sulfobromophthalein) and reactions with reactive products of P-450-catalyzed reactions (acetaminophen), to name just a few.

\[ R - X + GSH \rightarrow R - SG + XH \]  (i)

where X is a leaving group.

In the second type of reaction, a reduced substrate and glutathione disulfide (GSSG) are formed. In this second type of reaction, an unstable \( R - GS \) intermediate is the enzymatic product (Eq. ii) which is attached nonenzymatically by a second molecule of GSH, yielding the final product and GSSG (Eq. iii). Examples of substrates for this second type of reaction are organic nitrates and organic hydroperoxidase.

\[ R - X + GSH = R - SG + XH \]  ... ... (ii)

\[ R - SG + GSH = RH + GSSG \]  ... ... (iii)

Some forms (largely alpha class enzymes) also are steroid isomerases and GSH serves as a coenzyme rather than a substrate in this latter type of reaction (Douglas, 1987).

Many of the reactions catalyzed by the GST will occur in the absence of the enzyme. GSH is present in high concentration in many tissues and the relative importance of the catalysed vs. the nonenzymatic reaction in preventing cell injury is, as yet, unclear.
2.5.8. Monoamine Oxidase (MAO)

Monoamine oxidase is a flavin-containing enzyme located on the outer membrane of the mitochondria (Costa and Sandler, 1972). Oxidative deamination of primary monoamines by the mitochondrial enzyme monoamine oxidase (MAO, EC 1.4.3.4) produces NH₃, aldehydes and H₂O₂, agents with established or potential toxicity (Cooper et al., 1978; Benedetti and Dostert, 1989). The following reactions are catalyzed by MAO:

\[
RCH₂CH₂NH₂ + O₂ + H₂O \rightarrow RCH₂CHO + H₂O₂ + NH₃
\]

MAO is one of the major mammalian neuronal enzymes. It is active in both neurons and glial cells in the brain. MAO plays a strategic role in inactivating catecholamines that are free within the nerve terminals and not protected by the storage vesicles (Coyle and Snyder, 1981). When monoamines leak from the synaptic vesicles, MAO acts within the nerve fibre itself. The enzyme serves to oxidize some of the 5-HT, DA and NE after their release into the synaptic space in the nervous system, thus terminating their action. The concept of two functionally distinct forms of MAO has gained wide acceptance (Johnston, 1968; Houslay et al., 1976; Leung et al., 1981). Type A deaminates neurotransmitter amines such as 5-hydroxytryptamine (5-HT) and noradrenaline (NA) and is inhibited specifically by glergyline [N-methyl-N-propargyl-3- (2,4, dichlorophenoxy) propylamine], whereas type B oxidizes benzylamine and β-phenylethylamine and is preferentially inhibited by deprenyl phenylisopropylmethylpropylnamine (Tipton and Della Corte, 1979). Both forms deaminate substrates such as tyramine and tryptamine (Houslay et al., 1976).
The MAO-B activity increases in human brain in ageing (Robinson et al., 1972), while the MAO-A activity either increases (Shih, 1979) or remains unchanged (Fowler et al., 1980). In old rats, MAO-A activity was decreased significantly in all the regions studied except in the cerebellum, where it was unchanged. On the other hand, MAO-B activity increased in all the areas studied except in the brain stem, where it decreased (Leung et al., 1981; Danh et al., 1989). Thus the regional changes in MAO-B activity were not dependent on those in MAO-A activity. This suggests that the mechanisms which alter the activities of the two forms are not related with each other. Any change in the usual amine concentration will disturb their activities and results in convulsive seizures (Kilian and Frey, 1973). Hence, it is likely that ageing and stress may also influence the monoamine concentration in the brain and might also be acting through this mechanism in producing central toxic effects.

2.5.9. Nucleic Acids and Nucleases

In the Central Nervous System (CNS), nucleic acids are characterized by their size, composition and role in protein synthesis. In the brain the nucleic acids (DNA and RNA) provide for the storage and transmission of genetic information as well as translation of this information leading to the synthesis of cellular proteins (White et al., 1978).

A knowledge of DNA helps in understanding the tissue components such as average cell densities, dry weight/average cell and total number of cells in each brain area (May and Grenell, 1959). Since the majority of cells in brain are diploid, there is generally a fixed quantity of DNA per cell (Heller and Elliott, 1954). The amount of DNA in white matter
approximately equals that in the cortex, and regional differences in the amount of brain DNA are relatively small (Bodian and Dziewiatkowski, 1950; Logan et al., 1952; Elliott and Heller, 1957). However, only cerebellum shows exceptionally high amounts of DNA (Mihailovic et al., 1958; Palladin, 1955; Grenell, 1958; May and Grenell, 1959).

DNA seems particularly plausible as a critical target in ageing because of the central role of DNA in information transfer between generations of somatic cells. Burger (1957) found parallel changes of DNA in brain with age. The low point for DNA is in the third decade, the time at which the brain reaches its greatest weight. From then on, DNA increases so that in the group of patients 30-90 years of age it is higher than at any other time in the life-span. As the dry weight of the brain decreases steadily from the third decades of life through the ninth, it is important to estimate if this apparent increase is a real one in terms of the total amount of DNA present in the brain. To answer this question, the weight in grams of dry matter on the basis of the average size brain for each decade of life was calculated. On this basis it was found that the total amount of DNA was increasing in the brain. The data suggested that in the ageing brain, although there was loss of protein and lipid, there might be at the same time an increase in DNA which might be imputed in part to a proliferation of the glial elements. Burger (1957) suggested that the increase in DNA in old age is due to two factors: an increase in pyknosis of the neurons and growth of glial elements. These data from Burger add to those of Hyden (1955) who found a decrease in DNA in the cytoplasm of brain cells from aged animals.
An enzyme called deoxiribonuclease breaks down DNA. In infant as well as adult rat brains an acid and an alkaline DNase have been identified (Sung, 1968). Out of the two enzymes, alkaline DNase shows a strong preference for denatured DNA, while the acid DNase prefers native DNA. In the senescent rat brain, DNase activity was found to be twice as high as that in young adult rat brain (Asano et al., 1979). With regard to cellular localization, neuronal nuclei prepared from young and adult rat brain have been shown to have a higher DNase activity than glial nuclei (Stambolova, 1973). From our laboratory, Tayyaba et al. (1981) reported that there was a remarkable decrease in the DNA level in all the brain regions studied after 'metasystox', (an organophosphate pesticide) toxicosis. On the other hand, the activity of DNase was found to be increased.

Study of RNA is very helpful in knowing the rate of protein synthesis and also in understanding the functional status of the nervous tissue (Bergen et al., 1974). The amount of RNA in gray matter usually exceeds that in white matter (Bodian and Dziewiatkowski, 1950; Logan et al., 1952; Mihailovic et al., 1958). RNA is highly concentrated in the nucleolus and in the Nissl substance of the cytoplasm of nerve cells (Caspersson, 1936, 1940; Landstrom et al., 1941). The RNA concentration has also shown variations within different brain regions, the highest concentration being in cerebellum, hypothalamus and cerebral cortex and the lowest in medulla (May and Grenell, 1959). Several investigators have observed that there is a proportionality between RNA content and the surface area of the cell body. The perikarya contain more RNA than axons.
In vertebrates, extracellular RNase is secreted by the pancreas and salivary glands (Ellem and Colter, 1961). The usual function of RNase is, obviously, to break-down RNA.

The contention that nerve cells are lost with age is common, although reliable data showing that ageing is always associated with cell loss are difficult to find. Colen (1972), in a study of human brain, found a 44% loss of neurons at the age of years, which in itself would lead to a loss of RNA.

Hyden (1964) analysed the anterior motor horn cell from human spinal cord with the single-cell technique of Edstrom and found an average of 670 pg in 40 to 50 years old men and only 540 pg in 60 to 70 years old men. Mann et al. (1977) measured RNA absorption at 260 nm in the brain of 82 persons of age range 2-91 years. They found a decrease of only about 15% in the neurons of the hippocampus and the dentate nucleus at the age of 90 years. The olivary neurons, however, showed a loss of RNA of about 60%. The nucleolar volume showed a reduction of similar magnitude. Lipofuscin pigment increased in a reverse proportion, a finding for which they had no explanation. Thus, it might be stated that although RNA content decreases with age in most areas, this decrease is by no means synchronous in all regions of the brain.

The brain and nervous tissue contain several RNases distinguished by different pH optima and present in free or latent form (Koenig et al., 1964; Ghosh and Ghosh, 1969; Takahashi et al., 1970; Guroff and
These RNases are thought to be involved in regulating either the processing or the cellular amount of RNA. It seemed interesting, therefore, to determine whether the RNase activities in some CNS regions of the rat are affected by ageing. Decreased cellular RNA content, a consequence of neuronal death in the senescent brain, is a phenomenon which, inter alia is associated with the ageing process (Von Hahn, 1981). The level of the inhibitor factor of alkaline RNase has been shown to decrease with age of the animal in various tissues of the rat, such as blood lymphocytes, liver, thymus, and lymph nodes, although total RNase activity was found to be higher, especially in the lymphoid organs of older animals (Kraft and Shortman, 1970a,b). The activity of liver acid RNase increased slightly in aged mouse, although it remained almost unchanged in hamster and guinea pig (Goto et al., 1969). No age-related changes in free alkaline RNase activity of postmitochondrial supernatant of rat forebrain have been reported (Dwyer et al., 1980); however, a sharp decrease of the same enzyme activity in the whole brain of old rats has also been found (Ekstrom et al., 1980).

2.5.10. Protein

Protein is one of the important biochemical components of the brain in vertebrates. It constitutes 40% of the dry weight of the whole brain and 8% of the weight of whole fresh brain (McIlwain and Bachelard, 1971). The gray matter contains larger amount of protein than the white matter. This difference probably affects the large volume of tissue occupied by myelin sheaths in white matter. Proteins are present in
the brain in steady-state. There is a high rate of metabolic activity in the brain. this view is well-correlated with the presence of large amount of cytoplasmic ribosomes which provide large number of sites for protein synthesis (McIlwain and Bachelard, 1971). Any change in protein concentration may influence the metabolic rate of tissue. It requires rapid synthesis and renewal of protein. According to Hyden and Lange (1972) increased neuronal activity decreases or inhibits the synthesis of protein. Proteins extensively mediate the specific neuronal functions, such as conduction of action potentials and synaptic transmission (Bock, 1978).

A number of brain specific proteins have been detected by their antigenicity and their developmental changes have been studied (Bock, 1977; Jaque et al., 1976). Age-related declines in the rates of protein synthesis are widely described in brain and other rodent organs (Dwyer et al., 1980; Rothstein, 1982; Richardson et al., 1987). In mouse brain, the concentration, which is very low until day 7, increases rapidly to the adult level by day 23. The antigen is primarily in gray matter, and its pattern of increase correlates with synaptogenesis (Acton and Pfeiffer, 1978). Glial fibrillary acidic protein (GEAP), localized in astroglial processes (Bignami and Dahl, 1974), shows a peak concentration at 10 to 14 days in mouse brain, reflecting astroglial differentiation to the time of myelination (Jaque et al., 1976). Changes in the protein composition of isolated myelin fraction have been demonstrated with increasing proportions of basic and proteo-lipid proteins relative to proteins of higher molecular weight.
There is general agreement that rates of ribosomal-directed protein synthesis decrease with the maturation of neural tissue, but the factors responsible for the decline in activity have not been defined (Oja, 1973). Some investigators have found that ribosomal activity measured 'in vitro' decreases with maturation, whereas others have not. Perhaps the discrepancies are caused by variations in methods of ribosome preparation in the source of mRNA, or in incubation for measurement of protein synthesis. No major structural differences have been detected in brain ribosomes for young as compared to old animals. Possible control mechanisms under investigation include levels of specific mRNAs, which may be the single most important influence for regulation. Changes occur in soluble factors, capacity of ribosomes to bind these factors, and chemical modification of ribosomal proteins, for example, by phosphorylation. In addition to decreasing rates of protein synthesis with development, the average half lives of protein become greater, indicating decreasing rates of breakdown. The activity of brain proteinases which increase just after birth, decreases with maturation, in parallel with the increasing half-life (Marks and Lajtha, 1970).

2.5.11. Cortisol

Cortisol is a corticosteroid hormone, which is secreted from the adrenal cortex. The hormones of the pituitary-adrenocortical axis are involved in the regulation of functions of the central nervous system (Holsboer, 1989). They not only coordinate the neuroendocrine processes to stress itself, but also affect psycho-physiological processes. In 1936, Selye observed that diverse noxious agents cause an enlargement of
the adrenal cortex as a consequence of the "stress syndrome". Yates and Maran (1974) reported that a variety of stressful events cause a release of ACTH from the anterior pituitary. The secreted ACTH stimulates the synthesis of corticosteroids in the adrenal cortex. The elevated corticosteroid levels in plasma then inhibit the further release of ACTH from the pituitary. In a series of elegant experiments, Harris (1948) demonstrated that the release of ACTH from the pituitary is regulated by a corticotropin-releasing factor (CRF) from the hypothalamus. The CRF is synthesized in the hypothalamus. The CRF synthesized in the hypothalamus reaches the pituitary by a private portal blood supply. It then stimulates the secretion of ACTH from the pituitary (Fig. 2.9). After a long period of intensive investigations, CRF was isolated and purified, and its structure was characterized as a 41 amino acid peptide by Vale and co-workers (1981). CRF was thought to be the major, if not the sole means, of releasing ACTH from the pituitary. ACTH can also be released and regulated by catecholamines and other hormones (Axelrod and Reisine, 1984).

There have been a number of investigations using cortisol to assess the reaction of the pituitary-adrenocortical axis under various conditions. Lundberg and Frankenhaeuser (1980) found cortisol levels increased in situations which were accompanied by boredom, impatience and tiredness (vigilance task). In situations characterized by a high controllability and predictability (self-placed RT-task), Lehmann et al. (1992) reported an adrenocortical suppression. Furthermore, there is increasing evidence that cortisol modulates brain function in humans. This principal endogeneous glucocorticoid in humans increases slow-wave sleep and decreases rapid-
Fig. 2-9. REGULATORY MECHANISM FOR CORTISOL.
eye-movement sleep (Born, et al., 1991). There is some evidence that heart rate changes are accompanied by cortisol changes dependent on personality. Furthermore, an increasing heart rate is related to increasing difficulty of a task (Eason and Dudley, 1971; Carroll et al., 1986).

2.6 Lacunae in knowledge:

A critical survey of the literature revealed that:

i) Lipids are essential components of all cellular structures in the brain and the level of lipids is altered during ageing as well as stress. Regional studies of lipids in the CNS are relatively limited in number. The effect of restraint stress on lipids of the various regions of the brain is unclear.

ii) Lipid peroxidation is one of the major causes of ageing. It is a free-radical-mediated chain reaction. During stress, reactive oxygen species are readily generated. No study could be traced where the effect of restraint stress on the products of lipid peroxidation, such as lipid peroxides, lipid hydroperoxides, conjugated dienes and lipofuscin was studied.

iii) Reactive oxygen species or oxygen-centered radicals damage the cell. A precise nature of oxygen radicals produced in the CNS was by no means clear. The presence of diffusible antioxidants provides protection against free radicals. Glutathione is essential for the protection of cell and protective enzymes such as SOD, CAT, GR, GSHPx and GST, are responsible for defense against free radical induced damage. There was no study traceable to evaluate the effect of stress on these enzymes in the aged rats.
iv) Effect of passage of time on the levels of monoamine oxidase (MAO) has been studied. However, investigations related to the effect of restraint stress on the MAO are lacking.

v) Studies have been conducted to see the effect of senescence on the nucleic acids (DNA and RNA) and nucleases (DNase and RNase). DNA is central to genome and RNA is responsible for protein synthesis. However, the effect of restraint stress on regional distribution of nucleic acids and nucleases was not studied in aged rats.

vi) Protein is one of the major constituents of the cellular organelles and biomembranes. Protein damage and an increased role of intracellular proteolysis in aged animals have been subject of critical study. However, we could not find any such study where the effect of restraint stress might have been studied in aged rats.

vii) Cortisol is one of the "markers" for stress. During stress cortisol level is highly affected. However, information on the effect of restraint stress on cortisol level in aged rats was not available in the literature.

viii) N-acetylhomocysteine thiolactone (citiolone) is a -SH group donor drug. The effect of citiolone on various regions of CNS in aged animals following restraint stress was not studied to date.

ix) Scanty studies have been undertaken to characterize the structure and function of various cellular organelles whose morphology might be altered during ageing and chemical stress. Also there was no information available on the effect of restraint stress on the alterations in morphology and ultrastructure of cellular components following restraint stress.
2.7 The Scope of the Present Study

Industrialization and urbanization are the result of advancement in various fields of science and technology, but they create many health hazards and diseases. Simultaneously, great progress was made in medical sciences which increased the life expectancy. With the success of the family welfare programme in India, the percentage of aged individuals is gradually increasing in the families with the limitation in the number of children. The process of ageing is multifactorial and it involves componently from biomolecules to the whole organisms, the environment and the society as a whole. The environmental stress is the scourge of the present civilization and man has to pay for his progress. Various types of stressors are known - physiological, biological, chemical, psychologic-al, economical etc. With the passage of time, restriction of activity of the aged individuals occurs. This can be simulated by experimental restraint stress for the purpose of studying the stress alterations in various regions of brain of the experimental animals. The existing knowledge in relation to human health, as far as ageing is concerned, is mostly based on observational study, as experimental studies are very difficult to perform on human beings. However, it is an established fact that human beings undergo certain specific structural and functional changes with the passage of time and certain diseases, for example, certain cancers, chronic and degenerative diseases etc. are known to occur more in elderly population.

It is known that stress of any type is liable to affect adversely the health of individuals physiologically, anatomically, and behaviorally.
As physical activity generally tends to decrease in the elderly, either as a cause or effect of ageing phenomenon, it will be interesting to know whether or not immobilization produces any change in the various regions of brain. Immobilization is a type of stress. In younger generation also, stress may cause various types of diseases.

Since brain is the source of the events which finally govern the behaviour of an organism, any impaired activity or ability of various nerve cells to function normally may stem from stress. There may be biophysical, biochemical and/or anatomical lesions in a particular part of the nervous system. Such changes in the structure and chemical composition of brain regions will manifest merely as signs of damage. This, in turn, would help to understand organisms' adaptive processes for maintaining behavioural, autonomic, endocrine, and metabolic responses to preserve homeostasis. A study of the stress-induced alterations in the brain will constitute one of the most exciting facets in intensive multi-disciplinary stress research. Regional biochemical and morphological alterations will provide useful information. Furthermore, stress studies by accelerating changes normally occurring in the organism with the passage of time (ageing), will facilitate better insight into the understanding of the otherwise slow and subtle changes occurring due to wear and tear. The understanding of "brain mechanisms" will facilitate a more rational therapy of not only neurological and psychiatric disorders but also disorders of various organ systems controlled by the brain. However, despite some notable progress in understanding of the "brain mechanisms", the field is still wide open for an integrative frame-work which can explain the majority of research results in a logical and theoretical manner.
It must however, be borne in mind that the brain is constructed in such a complex manner that analysis of whole brain, or large samples there of, may give little indication of the special function and metabolism of its different regions.

Keeping in view the limited information on the subject under discussion, our study is likely to open up new vistas regarding the comparative effects of ageing, stress and antioxidant, N-acetylhomocysteine thiolactone (citiolone) on neurochemical and neurohistological mechanisms in different brain regions of the rat.