CHAPTER - IV

DISCUSSION

D I S C U S S I O N

In the present study age related changes in the activity and kinetics of acid phosphatase in the liver of male mice have been worked out. Total enzyme activity/gm in tissue homogenate with or without Triton X-100 was low in juveniles increased in adult and further decreased in old. In microsomal and lysosomal fractions enzyme activity/gm. was estimated, which also showed similar pattern as above. When microsomal and lysosomal enzyme activities/gm inbetween the groups were compared, microsomal enzyme activity was less in juveniles but more in adult and decreased in old. Similarly lysosomal acid phosphatase activity was low in juvenile, increased in adult and decreased in old. When enzyme activities were estimated on the basis of mg/protein, in total homogenates, activities were more or less same in juveniles and adult, but increased significantly in old, When microsomal and lysosomal acid phosphatase activities/mg protein were compared the picture was somewhat different. In juveniles microsomal activity was less in comparison with the adult, which was further increased in old. On the contrary lysosomal activity was high in juvenile and decreased in adult and old, thus the microsomal to lysosomal ratio was low in juveniles and increased in adult and old. In conclusion we claim that in total homogenate there was decrease in acid phosphatase activity in old whereas microsomal and lysosomal enzyme activities/mg protein showed

reverse relationship the microsomal activity was increased in aging and lysosomal was decreased. In 1970 Guder and his colleagues showed change in the ratio of free to total activity of lysosomal enzyme in rat liver in aging. Platt (1970) showed decreased inducibility by phenobarbital with aging for the lysosomal enzyme β-glucuronidase and β-N-acetyl glucosaminase in rat liver. Van Mahen and his colleagues (1983) in their studies showed that the activity of lysosomal enzyme was higher in peripheral area and reverse situation in the central area ofliver. They in 1984 (De-Priester et al, 1984) further showed that the lysosomes in the central area were in the form of dense bodies including lipofuscin, containing in senescent animals. These observations indicate the formation of nonfunctional lysosomes during aging. Tanaka et al. (1987) showed marked decrease in the lysosomal enzyme activity as rat grows older. This they have observed in lysosomal fraction. Nakano and Mizunc (1989) isolated rat hepatocytes and separated cells according to ploidy classes. The ploidy was increased during aging. Number of octaploid cells were more in old than young and hydrolytic enzyme activity was greater in higher ploidy classes than lower ploidy class hepatocytes. In their study they have not paid attention to microsomal and lysosomal forms of the enzyme. Serafinska et al. (1988) studied acid phosphatase activity at different time intervals in rat liver at different age groups. They have related the change with circadian rhythem, but when their

observations were carefully studied indicate the increase in enzyme activity in old liver. They have taken total homogenate for their study. Ferland et al. (1990) showed increase in lysosomal enzyme activity in homogenates, kuffer cells and endothelial cells isolated from the livers of female rats.

Age related fluctuations in the activity of lysosomal enzymes in different cells and tissues have been subject of several studies. The examination done on lysosomal enzyme studies during aging brought contradictory results.Studies with other organs except lymphocytes (Moszcsynski and Lisiewicz, 1983; Mysliwska et al., 1985 a,b, - - - Lisiewicz and Mosczynski, 1985; Macejka et al., 1990) and Nakano et al. (1985) in the kidney, in brain (Constantinescu, 1981; Aoyagi et al. 1986; Nakamura et al.,1989 a,b; Jadhav, 1990), in retinal pigment epithelium (Swanson, 1966; Gorthy et al., 1971; Gorthy, 1989; unakar et al.,1977, 1982, 1985; Penn et al.,1985; Katz and Robinson, 1984; Hayasaka, 1989; Wyszynski, et al., 1985; Weinreb et al., 1991); in blood vessels (Kirk, 1962, 1969; Buddrecke et al., 1969; Clair, 1976; Sasahara et al., 1988), in heart (Strehler et al., 1959; Reichel, 1968; Comolli, 1971; Travis and Travis, 1972; Tomanek and Karlson, 1973; Wildenthal, 1977; Trauring and Papka,1980; Decker andWildenthal, 1981; Salminen and Vihko 1981; Salminen et al.,1985; Schmucker and Sachs, 1985; Kurane, 1992), in thyroid (Neve and Rondeaux,

1991), in murine vertebrae (Bar-Shira maymon et al.,1989) in muscles (Gutmann et al.,1976); in prostrate (Vasilenko and Sergienko, 1983) there was increase in the lysosomal enzyme activity. Some studies reported that the lysosomal enzyme activity do not change in parallel with aging. Hinkle (1988) in prostate Nakamura et al. (1989b) in brain; Nimbalkar (1991) in salivary glands and Weinreb (1991) in aqueous humor.

The discrepancies could have been due to the use of various parameters for the expression of acid phosphatase activity in wet weight, protein, nitrogen and DNA content. Age dependent change may also be the characteristic of different tissues and nature of lysosomal enzyme. We have seen from the review on lysosomal changes during aging in the introductory chapter that the activities of cathepsins are increased in heart brain and muscles, acid phosphatase in liver and prostate gland and glycosidases in blood vessels.

Our conclusion is that, it is necessary to study lysosomal enzymes in different cell fractions and also on the basis of specific activities. We have studied acid phosphatase activity in total homogenates, total homogenate with Triton X-100, microsomal and lysosomal fractions, we found increase in enzyme activity in adult compared to juvenile and decrease in old. In Triton X treated samples there was apparent increase in the enzyme activity in both the samples but no change in the behaviour of the enzyme was

in old, where decrease in enzyme activity was noticed. In the study of enzyme from different fractions on the basis of /mg protein, in juveniles microsomal enzyme activity was low and increased in parallel with aging, vice-versa was the behaviour of the enzyme from lysosomal fraction. The microsomal acid phosphatase shows pH optimum above 5 pH and the lysosomal acid phosphatase shows below 5 pH (Dipietro, 1968; Dipietro and Zengerle, 1967). In our study microsomal acid phosphatase also active below pH 5 and also above 5 pH. This shows that the microsomal fraction also consisted of lysosomal acid phosphatase. This may be due to the association of cytosol acid phosphatase leaked from lysosomes and therefore there must apparent increase in the acid phosphatase activity in microsomal fraction. Studies on protein estimations in tissue homogenates neither showed significant increase nor decrease in the protein content but there was increase in microsomal proteins and decrease in lysosomal fraction proteins, this supports the view that lysosomes loose their protein content and consequently enzymes in aged mice. In the present study it has also observed that there is positive correlation between malonic dialdehyde formation and a microsomal acid phosphatase activity and negative correlation in case of lysosomal acid phosphatase. Lysosomal instability in aged brain has been described by some workers.

Nakamura and his coworkers (Nakamura et al., 1989 b).They showed increased activity for cathepsin D and β-glucuronidase in cytosolic fraction. Insufficiency of lysosomal functions in aged subjects was observed De-Duve and Beauty (1959) and Slater (1968).From our study and others general hypothesis can be made that in the liver atleast lability of lysosomal membrane with subsequent release of hydrolytic enzymes into cytosol leads to insufficiency of lysosomal functions. According to Moszczynski (1983) acid phosphatase +ve lysosomal apparatus in lymphocytes demonstrates a marked stability from neonatal period upto fifth decade of life and onlythe last decades of life i.e. in between 66 and 90 years there is destabilizations of lysosomes which is manifested by a diminished number of cells having no intact lysosomal granules and showing diffused enzymatic reactions reflecting the transfer of enzymatic content of lysosomes into the cytoplasm. They concluded that the observed changes probably correspond to the aging of lymphocyte system and consecutive diminishing of immunity in aged subjects. In the present investigation we observed that there was increase in the ratio of microsomal/lysosomal enzyme activity whichmight have been due to the transfer of enzymatic content of lysosomes into cytosol. Oxygen free radicals including (O_2) and hydroxyl radicals (O.H.) have been suggested to exert their cytotoxic effect by causing peroxidation of membrane phospholipids which

can result in increase in membrane fluidity, increasing permeability of membrane and loss of membrane integrity (Freeman and Crapo, 1982; Halliwell and Gutte-ridge, 1982; meerson et al., 1982). Tissue ischemic triggers a variety of responses including increased fragility of lysosomes and release of lysosomal acid hydrolases (Brachfeld, 1968; Spath et al., 1974; Wildenthal, 1978; Kalra et al., 1988), which further lead to insufficiency of lysosomal functions which may cause increase in lipofuscin granules during aging.

The formation of lipofuscin granules linked with old age due increase in lipid peroxidation of the membranes in old so much that the lysosomes unables to digest the membrane waste (Chio and Tappel, 1969a,b; Donato & Sohal, 1981; Patro, 1985, Patro et al., 1987, 1988, 1989). But now the view that cells get loaded with lipofuscin granules even in very young age if lysosomal system is disturbed. Lysosomal associated lipofuscin like particles are observed if lysosomal enzymes are inhibited by chloroquine (Sharma et al., 1987), leupeptine (Ivy et al., 1984), fenfluramine (Thakkar et al.,1990). Inhibition of lysosomal enzymes or insufficiency of lysosomal enzymes may disturb equilibrium between formation and elimination rates of lipofuscin.

Insufficiency of lysosomal function may also due to the change in the kinetics of the lysosomal enzymes. The larger

the Km, the enzyme has less affinity for the substrate which further affect the enzyme activity. This may be due alteration in the enzyme molecule. Change in the enzyme molecule could also be judged by the study of effect of temperature and heat inactivation of the enzyme at higher temperature and effect of inhibitor on the Km of the enzyme activity. Acid phosphatase of both fractions of old showed increase in the pH optima compared to adult, more inhibition of the enzyme molecule was observed in temperature and heat inactivation study. Inhibitor L+ tart-rate affect the Km in old more than adult. Wyszynski (1989) studied donar age dependent change in the activity of acidic glycosidase and acid phosphatase in cultured human retinal pigment epithelium with regard to Km, pH, Vmax, but found no change.

In conclusion it can be said that -

1) In aging process there is apparent loss in acid phosphatase activity in old mice.
2) Microsomal fraction shows an increase in the enzyme activity whereas in lysosomal it shows a decrease in the liver of old male mice.
3) There is no significant change in protein content in old but there was difference in lysosomal and microsomal fraction, in microssomal fraction the protein content is high.
4) There is positive correlation between malonic dialdehyde formation and microsomal enzyme activity, and negative

correlation between malonic dialdehyde formation and lysosomal enzyme activity.

Irrespective of precise underlying mechanisms, the present data suggest that age related changes in the activity of enzymes may play some role in the process of aging

5) It is suggested that due to lipid peroxidation there may be destabilization of lysosomal membrane which leads to the leakage of lysosomal enzymes in cytosol this makes lysosomes weak in their function which may results in the formation of lipofuscin granules.

6) It has also been suggested that malonic dialdehyde makes enzyme molecule inactive by reacting with amino group resulting in change in the kinetics of the enzymes, thereby lysosomal enzymes, which are supposed necessary to perform the autophagic process, are inactive which may cause accumulation of lipofuscin granules. The experiments in lysosomal enzyme inhibition associated with lipofuscin accumulation supports the foregone statement.