I N T R O D U C T I O N

INTRODUCTION

The biogenic amines are low molecular weight, aliphatic, non-protein nitrogenous bases that are widely distributed in biological materials. They are classified as mono-, di- and polyamines based on the number of amino groups they contain. The triamine spermidine and tetraamine spermine are structurally related to diamine putrescine, being derived by stepwise addition of propylamine residue to each amino group.

$NH_2(CH_2)_4NH_2$

1,4-diamino butane

(Putrescine)

$NH_2(CH_2)_3NH(CH_2)_4NH_2$

1,8-diamino-4-azaoctane

(Spermidine)

$NH_2(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$

1,12-diamino-4,9-diazadodecane

(Spermine)

Though, naturally occurring polyamines have been known since 1678, they were relatively unfamiliar to most investigators for quite some time. In recent years increasing evidence regarding their role in the metabolism of macromolecules, organelle structure and certain aspects of growth and development in various biological systems have caused a renewed interest in these compounds.

The occurrence, metabolism and functions of polyamines have been discussed in several reviews (Tabor et al., 1961; Tabor and Tabor, 1964; 1976; Cohen and Raina, 1967; Stevens, 1970; Smith, 1971; 1972; 1977a; 1980; 1981; 1982; Janne et al., 1978; Kaur-Sawhney and Galston, 1981; Galston, 1983).

Occurrence and metabolism

Polyamines are known to be ubiquitous in biological materials, though, their amounts may vary greatly in different cells. Presence of putrescine has been reported in algae (Kanazawa et al., 1966), fungi (List and Wagner, 1963) and several species of higher plants (Smith, 1977a; Palavan and Galston, 1982). Triamine spermidine was found in chlorella (Kanazawa et al., 1966), ustilago (List and Wagner, 1963) and along with tetraamine spermine in pollen grains of petunia

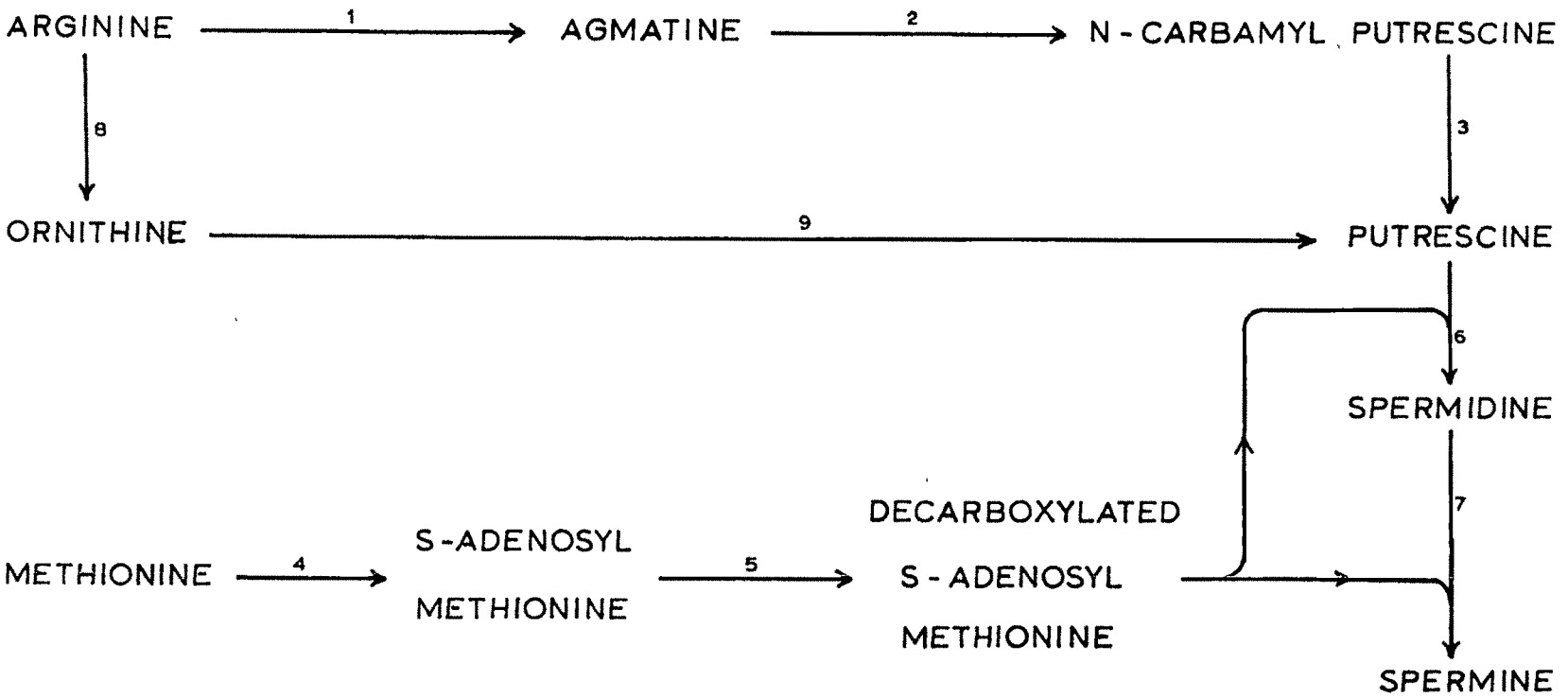
(Linskens et al., 1968), embryos of cereals (Moruzzi and Caldarera, 1964), seeds of a large variety of plants (Bagni, 1968) and in several other parts of plants (Bagni et al., 1967; Smith, 1977a; Palavan and Galston, 1982).

Cadavarine, a higher homologue of putrescine, was found in fungi (Umezu, 1970) and in higher plants (Palavan and Galston, 1982). 1,3-diamino propane, an oxidation product of polyamines, occurs in many cereals (Smith, 1981) and cucumber seeds (Flayeh et al., 1984). Some uncommon polyamines and their derivatives also occur in plants. Homo-arginine and homo-spermidine have been reported in the leaves of Santalum album (Kuttan et al., 1971), Lathyrus sativus (Ramakrishna and Adiga, 1974) and in root nodules of legumes (Smith, 1977b).

Di- and polyamines also occur as conjugates with alkaloids, steroids, glucosides and cinnamic acid and its derivatives in many species of higher plants (Smith, 1971; 1977a). In the leaves of Nicotiana tabacum, p-coumaroyl-, caffeoyl- and feruloylputrescine and caffeoylspermidine have been demonstrated on floral induction and these amides accumulate in considerable amounts in the reproductive organs (Cabanne et al., 1977). On infection with TMV coumaroyl-, dicoumaroyl-, feruloyl- and diferuloyl putrescine accumulate in hyper-sensitive lesions of Nicotiana tabacum (Cabanne et al., 1977).

Knowledge about the intracellular distribution of polyamines is scanty. Being basic substances they strongly bind to polyanions and are highly soluble in aqueous media which results in their re-distribution when the tissues are homogenized. It is, therefore, difficult to establish their precise location in the cell. Inspite of these difficulties spermidine and spermine have been shown to be present in both particulate and soluble fractions of etiolated pea epicotyls (Bagni and Fracassini, 1974).

Biosynthesis : Putrescine occupies a central position in the biosynthetic pathway of polyamines as it is the precursor of both spermidine and spermine. There are two pathways operating for the biosynthesis of putrescine (Fig.1). First is by decarboxylation of ornithine produced from arginine by arginase reaction. This pathway operates predominantly in animal systems (Heimer et al., 1979). The second occurs in plants, wherein arginine is first decarboxylated to agmatine (Ramakrishna and Adiga, 1975a; Smith, 1979) which is converted to N-carbamyl putrescine (Yanagisawa and Suzuki, 1981) and then to putrescine (Smith, 1965). In Sesamum indicum N-carbamyl putrescine is derived from citrulline by decarboxylation (Crocomo and Basso, 1974). Even though, agmatine pathway appears to predominate in plants, the presence of ornithine decarboxylase has been reported in several plant systems suggesting that both the pathways are operative in plants (Smith, 1977a;



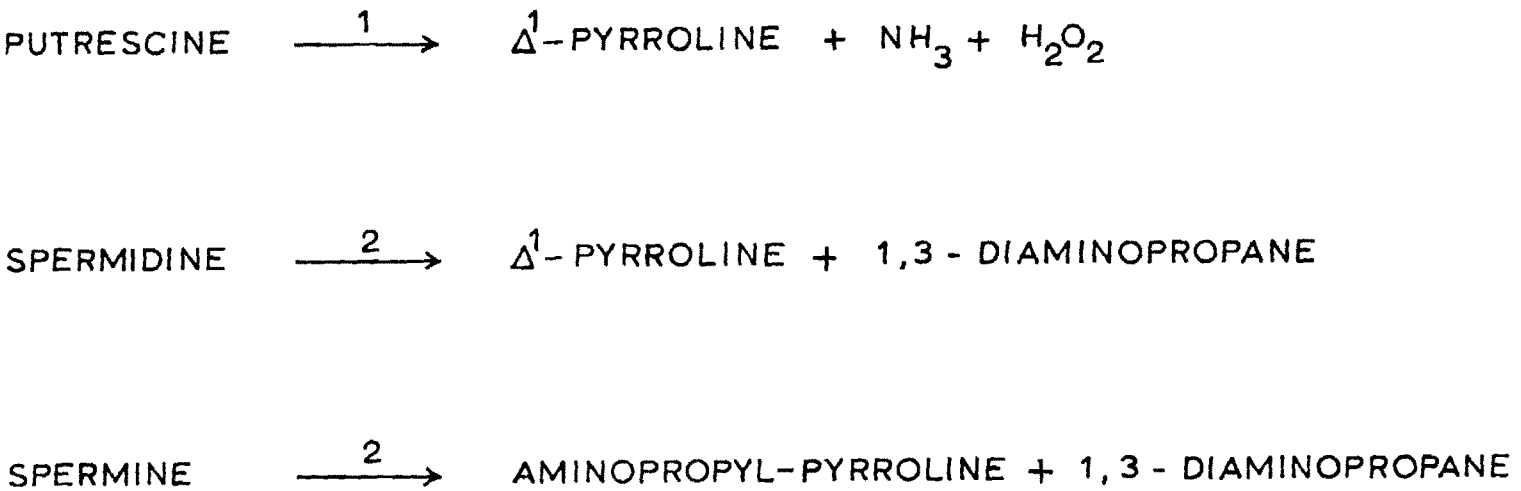
1. ARGININE DECARBOXYLASE
2. AGMATINE IMINOHYDROLASE
3. N-CARBAMYLPUTRESINE AMIDOHYDROLASE
4. S-ADENOSYLMETHIONINE SYNTHETASE
5. S-ADENOSYLMETHIONINE DECARBOXYLASE
6. SPERMIDINE SYNTHASE
7. SPERMINE SYNTHASE
8. ARGINASE
9. ORNITHINE DECARBOXYLASE

Fig.1. BIOSYNTHETIC PATHWAY OF POLYAMINES

Kaur-Sawhney et al., 1982a; Flores and Galston, 1982; Flores et al., 1984).

The two polyamines, spermidine and spermine are synthesized from putrescine by a stepwise addition of propylamine moiety derived from the decarboxylated S-adenosyl methionine which is formed from methionine (Suresh and Adiga, 1977). Addition of propylamine moiety to putrescine for the synthesis of spermidine and spermine is catalyzed by two separate synthases (Cohen et al., 1981). The enzymes for both the pathways have been well characterized (Smith, 1980).

Degradation : Major pathway for the degradation of the polyamines is by oxidation, though, a number of other non-oxidative reactions such as transamidination (Kim and Tchen, 1962), carbamylation (Friedman, 1957) and transamination (Clark et al., 1959) have also been demonstrated. Two types of oxidases have been reported (Fig 2), an unspecific amine oxidase, generally found in legumes, having high affinity for diamines, putrescine and cadavarine (Hill and Mann, 1968; Smith, 1977a; Matsuda and Suzuki,1981) and the other found in cereals, specific for spermidine and spermine, acting at the secondary amino group and forming 1,3-diaminopropane and pyrroline or amino propyl-pyrroline respectively from spermidine and spermine (Smith, 1971; Kaur-Sawhney et al., 1981).



1. DIAMINE OXIDASE
2. POLYAMINE OXIDASE

Fig. 2. OXIDATIVE DEGRADATION OF POLYAMINES

Polyamine levels under different growth and physiological conditions

Polyamine concentrations are reported to change during dormancy-break, mitosis, germination, reproduction, growth of cultured cells, embryogenesis, stress, infection, mineral deficiency, nitrogen nutrition as well as under the influence of phytohormones and light.

Starting from the last phase of dormancy polyamine levels increase during the sprouting of Helianthus (Torrigiani and Fracassini, 1979) and potato tubers (Kaur-Sawhney et al., 1982b). During first cell cycle after dormancy-break polyamine content increased during G and S phases and decreased as the mitosis proceeds (Fracassini et al., 1980). A correlation between the onset of mitotic activity and polyamine level has been reported in the embryos of Triticum durum, Helianthus annus and Glycine max (Anguilesi, 1980). Germination increased the polyamine concentration in Phaseolus (Bagni, 1970), Hordeum (Smith and Best, 1977), Triticum, Tragopogan, Zea, Lathyrus (Villanvera et al., 1978) and Oryza (Sen et al., 1981) seedlings. Highest polyamine content was found in the rapidly growing parts and lowest in the mature parts of the plants (Palavan and Galston, 1982).

Reproductive organs of the plant showed higher polyamine levels compared to the vegetative ones (Cabanne et al.,1977) and the levels increased further after pollination (Heimer et al., 1982). Polyamines tend to have their own gradients where cell elongation is the main growth process (Dumortier et al., 1983).

Polyamine levels increased in the rapidly growing Paul's scarlet rose cultures (Smith et al., 1978). Putrescine and spermidine levels were high in the habituated tumour tissue and in tumours induced by Agrobacterium tumefaciens compared to the normally growing tissue of Nicotiana glauca and Scorzonera hispanica (Bagni and Fracassini, 1979). Polyamine levels also increased when actively growing Daucus carota cells were transferred from nonembryogenic to embryogenic medium (Montague et al.,1978, 1979).

Putrescine level increased under stress conditions such as salinity (Smith, 1977a), SO_2 fumigation (Priebe et al., 1978), osmotic stress caused either by mannitol or partial wilting (Flores and Galstan, 1982; Flores et al., 1984) and low pH (Young and Galston, 1983). On infection with cuscuta reflexa polyamine levels decreased in the host plants whereas part of the parasite in contact with the host showed an increase (Naik et al., 1976).

Putrescine content has been shown to vary in plants grown in mineral deficient conditions. Under potassium deficiency putrescine level increased in Hordeum vulgare (Smith, 1973), Pisum sativum (Basso and Smith, 1973), Lolium multiflorum (Nowokowski and Byers, 1972) and Citrus (Bar-Akiva, 1975). In Hordeum it also increased under magnesium deficiency but decreased under phosphorus, sulfur and nitrogen deficiencies (Smith, 1977a). Along with putrescine the levels of agmatine, arginine, citrulline and N-carbamyl putrescine also increased in sesamum under potassium deficiency (Crocomo and Basso, 1974).

Polyamine levels were higher in Glycine max grown in the medium containing ammonia than the one containing nitrate or urea (Le Rudulier and Goas, 1971, 1979). Similarly, putrescine level was high in Nicotiana tabacum grown in ammonium sulfate. Addition of KCl decreased putrescine level in Nicotiana but increased cadavarine level in both (Hault et al., 1970).

Polyamine levels change on application of plant hormones. Their level increased in Helianthus tuberosus on application of indole acetic acid (IAA) (Cocucci and Bagni, 1968) and 2,4-dichlorophenoxy acetic acid (2,4-D) (Bagni et al., 1981a) and in rice on application of gibberellic acid (GA) (Mukhopadhyay et al., 1983).

But in dwarf pea seedlings only putrescine and spermidine levels increased on application of GA and decreased to control level on application of AMO 1618, a GA inhibitor (Dai et al., 1982). Putrescine and spermidine levels in cucumber seedlings (Suresh et al., 1978) and all the polyamines in rice (Mukhopadhyay et al., 1983) increased on kinetin application. Application of abscissic acid (ABA) decreased polyamine levels in rice (Mukhopadhyay et al., 1983).

Polyamine levels also change on exposure to different lights. Their level increased in the bud and decreased in the subepicotyl segments of pea on exposure to red light and the red light induced changes were reversed by far-red light (Goren et al., 1982a).

Role of Polyamines

(A) Growth and development : Polyamines have been described as growth factors in microorganisms, mammalian cell lines and higher plants (Cohen, 1971; Bachrach, 1973; Janne et al., 1978; Galston and Kaur-Sawhney, 1982). In plants they are implicated with various growth regulatory functions and are being considered as a new class of plant growth regulators (Galston and Kaur-Sawhney, 1982).

The effect of polyamines on plant growth was for the first time demonstrated in the dormant Helianthus tuberosus cultivated in vitro. Low levels of endogenous polyamines as well as plant hormones in the dormant tubers do not permit cell proliferation but when the polyamines were supplied exogenously the tuber cells were able to divide and grow without any other growth substance (Fracassini et al., 1980). The growth stimulation and vascular tissue arrangement was similar to that observed by treatment with IAA (Fracassini and Alessandri, 1982). The release of dormancy by plant hormone may be mediated by polyamines since it was shown that IAA and 2,4-D treatment increased the level of polyamines and their biosynthetic enzymes (Cocucci and Bagni, 1968). In potato tubers also it was shown that polyamine levels and their biosynthetic enzymes were high in the sprouted than in the dormant tubers (Kaur-Sawhney et al., 1982b).

Polyamines have also been associated with other processes such as seed formation (Bagni et al., 1967) and germination (Bagni, 1970; Ramakrishna and Adiga, 1975b; Smith and Best, 1977). Variation in polyamine concentrations during the seedling growth may be related to their function as growth factors. For example, during the growth of Phaseolus vulgaris seedlings, spermidine and spermine decreased in cotyledons and increased in shoots with

simultaneous and parallel changes in RNA and protein content (Bagni, 1970). Similar variations were also observed during later stages of growth (Palavan and Galston, 1982). Polyamine levels also increased during the growth of embryonic axis of Lathyrus sativus (Ramakrishna and Adiga, 1975b).

The increase in plant growth was associated with a parallel enhancement of arginine decarboxylase (ADC) and/or ornithine decarboxylase (ODC), the two key enzymes of the biosynthetic pathway of polyamines. For example, ADC increased in carrot tissue after transfer to an embryogenic medium (Montague et al., 1979), in tobacco cells grown in culture and in developing tomato ovaries after pollination (Heimer et al., 1982) or after auxin spray (Cohen et al.,1982). Polyamine biosynthesizing enzymes increased in pea internodes on application of GA which causes increased internodal growth (Dai and Galston, 1982). Even the red light increased growth in pea buds and decreased growth in stem segments is paralleled by increased or decreased ODC activity respectively, and this was reversed by far-red light (Dai and Galston, 1981). It has been suggested that the increased polyamine level due to red light was not due to the increased growth rate, but the increased growth rate results from increased level of polyamines (Goren et al.,1982b).

Explant growth in Helianthus was inhibited by canavanine (CAV) and canaline (CAL), the analogues of arginine and ornithine respectively as well as α-difluoromethyl arginine (DFMA) and α-difluoromethyl ornithine (DFMO) (Bagni et al., 1981b, 1982b). Similarly auxin induced growth in tomato ovaries was inhibited by DFMO which could be reversed by external application of putrescine (Cohen et al., 1982). Though, polyamines could not induce root initiation, the rooting induced by indole butyric acid (IBA) was inhibited by CAV and CAL (Friedman, 1982) indicating their involvement in rooting.

Inhibition of polyamine synthesis due to CAV during the cell cycle after dormancy-break paralleled the inhibition of RNA synthesis (Bagni et al., 1981b) indicating that the growth response mediated by polyamines may be because of their effect on macromolecules. Since, it was found that after dormancy-break, polyamine biosynthesis begins within 15 minutes whereas, nucleic acid incorporation was detectable only later. The increase in polyamine biosynthesis is a general phenomenon of cycling cells (Fracassini et al., 1980; Heby, 1981; Bagni et al., 1982a) and there is a temporal correlation between polyamine synthesis and the start of chromosomal replication which suggests that polyamines are involved in the preparation of nuclear DNA synthesis.

(B) Polyamines and macromolecules : Many of the roles of polyamines at molecular level are still not defined but evidences suggest that their role is to stabilize and organize DNA and RNA structures and activities (Zhurkin et al., 1980). Due to their polycationic nature, polyamines may bind strongly to nucleic acids. For example, inhibition of nucleic acid synthesis by actinomycin-D was abolished by spermidine, suggesting that polyamines have a binding site on DNA which can interfere with actinomycin binding (Bagni et al., 1971; D'Orazi et al., 1979). Attachment of polyamines to t-RNA was confirmed by their presence in t-RNAs isolated from pea epicotyles, spinach leaves (Cohen et al., 1969) and Helianthus tuber (Bagni et al., 1981a). Along with their binding to nucleic acids, polyamines are also known to be involved in nucleic acid synthesis. In oat leaf protoplasts they promote both DNA synthesis and inception of mitotic activity (Kaur-Sawhney et al., 1980). Spermidine induces RNA synthesis, notably of r-RNA and t-RNA in dormant Helianthus tuberosus (Bagni et al., 1971). It also stimulates nucleotide incorporation and RNA polymerase activity in maize seedlings (Stout and Mans, 1967) and in soyabean hypocotyls (Guilfoyle and Hanson, 1973).

Polyamines are also involved in protein synthesis. They could increase the rate of amino acid incorporation by ribosomes from dormant or activated Helianthus tuber tissue (Cocucci and Bagni, 1968) and in wheat germ system

(Igarashi et al., 1981). Addition of optimal concentration of polyamines resulted in the lowering of Mg^{++} requirement for protein synthesis (Cocucci and Bagni, 1968). Spermine could even replace the Mg^{++} requirement in the poly-U directed phenylalanine synthesis in barley ribosome cell-free incorporating system (Cohen and Zalik, 1978). These compounds could also increase the rate of polypeptide chain elongation but not the chain initiation (Sakai and Cohen, 1976).

(C) Senescence : The deteriorative processes which naturally terminate the functional life of an organ are associated with gradual encroachment of processes such as disintegration of chloroplast structure leading to loss of chlorophyll and photochemical activities, increase in the hydrolytic enzymes leading to break down of nucleic acids and proteins, increase in free radical formation which results in membrane disintegration and ethylene production (Thimann, 1980).

The involvement of polyamines in leaf senescence has been inferred mainly from the response to exogenous application of polyamines and their precursors. Early work with oat leaves (Von Abrahms, 1974) indicated that arginine, ornithine and lysine retarded senescence by inhibiting chlorophyll degradation and a net decrease in protein content. Arginine antagonises the senescence promoting effect of serine and somewhat lowered the protease activity (Shibaoka and Thimann, 1970).

Now it is clearly established that polyamines retard senescence in both attached (Altmann and Bachrach, 1981) and detached (Cohen et al., 1979; Kaur-Sawhney et al., 1982a; Altman, 1982a) leaves. Further studies have shown that 1,3-diaminopropane, an oxidation product of polyamines, is responsible for delaying the senescence (Shih et al., 1982; Fuhrer et al., 1982).

Cohen et al. (1979) have shown that senescing barley leaf discs can effectively retain the chlorophyll if they were placed in polyamine solutions, even though the PS I and PS II activities were decreased (Popovic et al.,1979). This may be due to the alteration of membrane lipids (Krupa and Baszynski, 1975) which might have resulted in the apparent destruction of chloroplast envelope. When Ca^{++}, which retards senescence, (Poovaiah and Leopold, 1973) was supplied before or together with polyamines, the ability of polyamines to retard senescence was decreased, but when polyamines were added first, Ca^{++} was found to have no effect indicating that the retardation of senescence by polyamines may be brought about by ionic mechanism (Kaur-Sawhney and Galston, 1979; Altman, 1982a). Contrary to their effect in dark, polyamines increased the chlorophyll breakdown in light. This may be a result of removal of magnesium from the chlorophyll which leads to its destruction either by direct photolysis or by an enzyme linked photo-decomposition (Kaur-Sawhney and Galston, 1979).

Kaur-Sawhney and Galston (1979) have shown that polyamines delayed or prevented the rise in protease and RNase activities along with the chlorophyll loss in dark incubated oat leaves. Similar results were also observed with radish leaves (Altman, 1982a). Though, the exact mechanism by which polyamines inhibit protease activity is not known, it was postulated that these compounds may either bind electrovalently to the enzyme protein, thus preventing the protease to act on protein (Kaur-Sawhney and Galston, 1979; Kaur-Sawhney et al., 1982c) or by inhibiting the de novo synthesis of protease (Shih et al., 1982). Contrary to these results Srivastava et al. (1983) have shown that polyamines did not have any effect on amino nitrogen content which is an index of protease activity. The inhibition of RNase by polyamines may be due to their binding to RNA so that less RNA will be available for RNase action (Galston et al., 1978; Kaur-Sawhney et al., 1978; Altman, 1982b).

Free radical induced lipid peroxidation which causes membrane disintegration has been reported to increase and superoxide dismutase to decrease during senescence (Dhindsa et al., 1981, 1982). Recent studies from our laboratory have shown that polyamines decreased lipid peroxidation and maintained the superoxide dismutase level (Srivastava et al., unpublished observation).

Since ethylene production was found to be inhibited by polyamines during the senescnce of apple fruit slices and protoplasts (Apelbaum et al., 1981), petals of Tradescantia (Suttle, 1981), oat leaves (Fuhrer et al., 1982) and in orange peel discs (Even-Chen et al., 1982), it was suggested that the antisenescent effect of polyamines may be by inhibiting ethylene production due to a possible competition for S-adenosyl methionine, a precursor for both polyamines and ethylene. Two possible explanations for the effect of polyamines on ethylene synthesis have also been provided. Polyamines may either inhibit the 1-amino cyclopropane-1-carboxylic acid (ACC) synthase, a pyridoxal phosphate linked enzyme (Yu et al., 1979), since they are known to bind and form a Schiff's base with pyridoxal phosphate (Keniston, 1979) or inhibition of membrane associated ACC converting system (Matto et al., 1981).

Further, since Ca^{++} was found to inhibit ethylene synthesis in apple fruit discs (Apelbaum et al., 1981) it was proposed that polyamines which bind ionically like Ca^{++} to the membranes may cause conformational changes in the membrane that may impair the functionality of ethylene synthesizing system. This hypothesis appears to be incompatible since polyamines stabilize membrane integrity

including ethylene production (Kaur-Sawhney et al., 1977). However, the contradiction may not be true if a specific microenvironment is required for ethylene biosynthesis (Matto and Leberman, 1977; Odawara et al., 1977) and polyamines may alter this microenvironment. However, polyamines could also reverse the senescence caused by exogenous ethylene indicating that some other mechanism must be operating for the retardation of senescence induced by ethylene (Galston, 1983).

While exogenous application of polyamines is necessary for understanding the senescence syndrome, endogenous changes in polyamines are clearly more significant. Altman and Bachrach (1981) and Srivastava et al.(1981) have shown that senescence is correlated with lower levels of polyamines in intact leaves which may be because of the decreased ADC and ODC activities (Kaur-Sawhney et al.,1982a). Contrary to this Birecka et al. (1984) have shown that plants having low polyamine concentration have a weak senescence syndrome and the ones with high concentrations have a strong senescence syndrome.

(D) Stress : Plants respond to various stress conditions by developing protective measures against them. Under stress conditions there is an activation of putrescine biosynthesis and a massive increase in diamine concentration. This occurs

21

whether the response is a long term one as in the case of mineral deficiency and high salt concentration or a short term one as in the case of osmotic stress and low pH. From the early studies of K^+ deficiency (Coleman and Richards, 1956) it was suggested that alkali-metal deficiency shifts the internal balance between the organic anions and cations in the direction of increased acidity. Similarly NH_4^+ nutrition, the uptake and assimilation of which leads to the H^+ production, causes an increase in ADC activity and putrescine concentration in pea, corn and wheat (Le Rudulier and Goas, 1979). Even in SO_2 fumigation the acidity increases as a result of the formation of HSO_3^-, SO_3 and SO_4 ions (Priebe et al.,1978) and in all these cases amine accumulation would function as a homeostatic mechanism to keep intracellular pH at constant level.

Putrescine concentration increases under thermal damage (Hasnain et al., 1980), wounding (Altman, 1982b) osmotic stress (Flores and Galston, 1982; Flores et al., 1984) and low pH (Young and Galston, 1982). The increase in putrescine concentration may be to provide protection to the plant against these conditions since externally applied polyamines could protect the plants in some of these conditions (Hasnain et al., 1980). They could also retain the percentage of germination in heat-treated seeds (Nezgovorova and Borisova, 1967), the survival of partially

imbibed mung bean seeds subjected to cryogenic storage (Altman et al., 1982). and could protect plants from chemicals and environmental factors such as frost, drought etc. (Okii et al., 1980). The protection provided by polyamines in these conditions may be by protecting or reversing the membrane disruption caused by these conditions which may be brought about by their interaction with SH groups of the membrane proteins (Naik and Srivastava, 1981) or because of their interaction with membrane lipids (Gary-Bobo, 1970).

(E) Membrane integrity and function : Arginine as well as polyamines were shown to stabilize oat protoplasts (Altman et al., 1977; Kaur-Sawhney et al., 1978). These compounds were also shown to stabilize oat protoplasts against lysis, induced by osmotic shock, by exposure to exogenous RNase and cell free supernatant fraction of mechanically lysed protoplasts (Galston et al., 1978). Polyamine stabilization of oat protoplasts may be due to stabilization of plasma membrane through surface binding and/or by direct involvement in macromolecular metabolism (Kaur-Sawhney et al., 1980).

Polyamines have been shown to reduce the betacyanin efflux from beet root discs (Naik and Srivastava, 1978; Naik et al., 1980; Hargreaves, 1980). Later it was found that polyamines reduce betacyanin efflux not only

from beet root discs aged under standard conditions but also in response to treatments which may enhance membrane damage such as ageing at high temperature, from freeze thawing and from treatment of discs with RNase and protease (Altman, 1982b). These compounds also reduced ion efflux from beet root and swede discs, beet and spinach leaves and apple cells grown in suspension cultures (Srivastava and Smith, 1982a).

Polyamines have been reported to modulate the activity of various membrane bound enzymes like glucose-6-phosphatase from rat liver (Johnson and Nordlie, 1980), ATPase from human erythrocytes and pig kidney (Peter et al., 1973), chick brain (Heinrich-Hirsch et al., 1977) and mitochondria of mung bean (Peter et al., 1981). They have also been shown to affect the phospholipid biosynthesis probably by affecting the acetylcholine esterase (Kossorotow et al., 1974; Peter et al., 1979).

The effect of polyamines on the membrane stabilization resulting in the regulation of membrane properties and membrane bound enzymes is generally believed to be associated with their binding electrovalently to the negatively charged phospholipids and thus bringing about a change in the surface charges of the lipid (Gary-Bobo, 1970). Some studies, however, also

indicate that apart from their binding to membrane lipids, polyamines may also interact with -SH groups of the membrane proteins (Naik and Srivastava, 1981; Srivastava and Smith, 1982a). This appears to be quite in agreement with the observation that interaction between protein and lipid is an important factor in regulating the membrane structure (Luttge and Higinbotham, 1979).

Guanidino Compounds : Guanidino compounds, which are structurally related to polyamines and are derived from arginine by transamidination, are present in plants (Reinbothe and Mothes, 1962; Bidwell and Durzan, 1975). Guanidine was reported from several higher plants (Guggenheim, 1958), agmatine from Panas tigrinus (Boldt et al., 1971), creatine and creatinine from cocoa (Bloch and Schoenheimer, 1941), guanidino acetic acid from Galegea officinalis (Reuter et al., 1969), guanidino butyric acid from a wide variety of plants (Guggenheim, 1958), octopine and nopaline from crown gall tumours (Lippincott et al., 1972) and canavanine from various legumes (Bell, 1960).

The structure of aliphatic monoguanidines strongly indicates that they should posses surface active properties. Guanidines have been shown to inhibit K^+ absorption by barley roots (Gomez-Lepe and Jimeniz,1975), cation and anion absorption by oat roots (Gomez-Lepe and Hodges, 1978), inhibit the energized transport of Na^+ and

K^+ in liver mitochondria (Gomez-Puyou et al., 1973), block the Na^+ channel in nerve and muscle membranes (Hillic, 1974) and increase the cell permeability in Allium cepa epidermal cells (Gomez-Lepe et al., 1979). Betacyanin efflux from beet root discs and ion efflux from the discs of beet and swede, spinach and beet leaf discs and apple cells grown in suspension culture were increased by a series of mono- and diguanidines (Srivastava and Smith, 1982a). A substituted guanidines hordatine present in barley seedlings has antifungal properties (Smith and Best, 1978). Dodine $NH_2-\underset{\underset{NH}{\|}}{C}-NH-(CH_2)_{11}-CH_3$ and guazatine

$NH_2-\underset{\underset{NH}{\|}}{CH}-NH-(CH_2)_8-NH-(CH_2)_8-NH-\underset{\underset{NH}{\|}}{C}-NH_2$ have been used as fungicides. The former was found to be active against apple scab and other foliage diseases (Byrde et al., 1962) and the latter against a wide spectrum of cereal seed borne diseases (Bartlett and Ballard, 1975).

Guanidines also inhibit growth and have antiproliferative properties (Fontanini and Scarpa, 1961). They inhibit the growth of Ehrlich's ascites tumour cells (Tomomatsu et al., 1975), etiolated barley seedlings (Gomez-Lepe, 1976), light and dark grown cress, barley and oat seedlings and apple cells grown in suspension culture (Srivastava and Smith, 1982b). These effects of guanidines may be by bringing about destabilization of the membrane.

Like polyamines, guanidines may also bind to the phosphate groups of phospholipids (Srivastava and Smith, 1982a, 1982b). This binding may occur at the same sites as polyamines, since it was found that guanidine could reverse the inhibition of betacyanin efflux caused by spermine (Srivastava and Smith, 1982a) and polyamines could reverse the growth inhibition of seedlings (Srivastava and Smith, 1982b) and antiproliferative property (Mihich, 1963) caused by guanidines.

Since both polyamines and guanidines bind with membranes and modulate its properties and functions it may be of interest to know if they modulate the two important membrane bound enzymes i.e. peroxidase and ATPase.

PEROXIDASE

Peroxidase (Donor : H_2O_2 oxidoreductase E. C. 1.11.1.7), catalyzes the oxidation of a wide variety of hydrogen donors in the presence of H_2O_2 (Gaspar et al.,1982). It can also oxidize several compounds like chalcones (Wilson and Wong, 1976), tyrosine and tyramine (Bayse et al., 1972) $NADH_2$ and $NADHPH_2$ (Akazawa and Conn, 1958), estradiol and thiols (Jellinck and Fletcher, 1971) and pyridoxal related compounds (Hill, 1970) in the absence of H_2O_2 .

Peroxidases are widely distributed in plants and are present in different organs (Macnical, 1966) and tissues (Van Fleet, 1959) of the plant. Within the plant cell peroxidase activity was histochemically demonstrated to be associated with different subcellular organelles (Poux, 1969). Subcellular distribution of peroxidase differs from tissue to tissue and some times from cell to cell.

Peroxidase exists in two forms within the cell, namely soluble and particulate. The particulate form is either ionically bound which can be easily solubilized by ionic solutions or covalently bound which can be solubilized only by means of digesting the plant material with the help of enzymes (Haard, 1973; Birecka and Miller, 1974).

Physiological processes mediated by peroxidase :

Peroxidases have been implicated in and associated directly or indirectly with various physiological processes. Peroxidase is involved in the release of dormancy (Gasper et al., 1973). Its level is higher in plants with shorter dormancy than those with longer dormancy (Poovaiah et al., 1972). In apple embryo, peroxidase increases with the removal of dormancy and germination increases it further (Thevenot et al., 1977). Its activity has been shown to increase during the germination of rice (Sengupta et al., 1977), corn (Dencheva and Klisourska, 1976), wheat

(Gaspar et al., 1977), oat (Ray and Biswas, 1970) and mung bean (Dendsay and Sacher, 1978).

The major role of peroxidase in germination may be by its reoxidation of $NADPH_2$ which is the rate limiting step for pentose phosphate pathway. The dormancy breaking effect of azide and hydroxylamine were found to be due to their inhibitory effect on catalase and thereby sparing H_2O_2 for peroxidase in the NADPH oxidase system. The source of H_2O_2 might be the result of glyoxysomal enzymes during the production of carbohydrate from fats (Roberts, 1973; Hendricks and Tylarson, 1974, 1975).

Peroxidase can also provide an alternate route for oxidation of NADH (Rubin and Ivanova, 1963) and it was suggested that mitochondrial peroxidases may constitute a secondary pathway for electron transport without coupling at the third site of oxidative phosphorylation. It was postulated (Rich et al., 1976) that the alternate pathway reduces oxygen partially to hydrogen peroxide and the peroxidase activity of mitochondria prevents its accumulation.

The physiological effects of peroxidase have also been ascribed to its control and participation in auxin catabolism which regulates the endogenous auxin level and thus the physiological processes depending on auxin. Considering that peroxidases are responsible for in vivo auxin

catabolism, it has been hypothesized that rooting and flowering are controlled by inverse variations of auxin level in their inductive as well as their initiative phases (Gaspar, 1981). Gilbert and Sink (1971) assigned to auxin a central role in the control of senescence. The activity of IAA oxidase and the level of peroxides and free radicals increased with senescence, some of which were involved in ethylene production (Beauchamp and Fridovitch, 1970). On the other hand, application of ethylene also enhances peroxidase activity and catalyzes the autocatalytic synthesis of ethylene by plant tissues. This phenomenon is common for both flower senescence (Mayak and Halevy, 1980) and ripening of climacteric fruits (Sacher, 1973).

Auxin has been shown to retard ripening of banana (Vendrell, 1968) and grape (Hale et al., 1970). In the latter, endogenous auxin declined at the time of initiation of ripening. Since peroxidase has the capacity for IAA breakdown it could provide a base for control of ripening by destroying endogenous IAA and thereby rendering the tissue sensitive to ethylene (Frenkel, 1972). Peroxidase activity increases with abscission also (Henry and Jensen, 1973). In this case the increase in peroxidase activity is related to decrease in auxin which occurs prior to abscission (Addicott, 1970) and increased lignification (Addicott, 1970).

The total peroxidase activity increases considerably during the passage of the cells from a state of division to elongation and differentiation. Different isoperoxidases are associated with different types of cell differentiation mostly through lignification (Alvarez, 1968), vascularization (Harkin and Obst, 1973) and fiber formation (Haard et al., 1974). It has also been suggested that the continuous increase in peroxidase activity in case of root, bud and flower formation is associated with a continued lignification process for xylem cell differentiation in the newly formed organs (Thorpe et al., 1978).

Plants are able to provide defence against pathogenic organisms during which they may be susceptible or resistent to pathogen. Peroxidases have been implicated in both the processes. Inoculation of plants with selected pathogens increased peroxidase activity (Stahmann and Demorest, 1972). The increased peroxidase activity may intensify the formation of lignin which in turn can prevent the penetration of pathogen (Vance et al., 1980). Peroxidase is also known to either kill the infected cells (Simons and Ross, 1971) or attack the pathogens (Venere, 1980).

Peroxidase activity often serves as a parameter in various stress conditions. It increases under UV

(Henry et al., 1979) x-ray (Chourey et al., 1973) and γ-ray (Wahid, 1980) irradations which may be due to the premature senescence brought about by irradiation (Van Huystee, 1968). Wounding also increases peroxidase activity either due to the formation of suberin as a protective layer on the wounded parts (Thomas and Delincee, 1979) or due to lignification (Rhodes and Wooltorton, 1978). The increased peroxidase activity due to temperature is related to a hardening mechanism in which low molecular weight phenolic compounds decrease while highly polymerized phenolics increase (Ranadive and Haard, 1972).

Peroxidase also serves as a parameter for drought (Chetal and Ninawatee, 1976), hypoxia (Siegal et al.,1966), salt stress (Stevens et al., 1978), ion status (Besford and Deen, 1977), mineral deficiency (Sajiv and Bar-Akiva, 1972) or toxicity (Mukherjee and Gupta, 1972) and air-pollutants (Horsmann and Wellburn, 1977; Endress,et al., 1980). In the above conditions changes in peroxidase activity are mainly dependent on the tolerance of the plant.

Phytochrome regulation of peroxidase activity has been reported in mustard (Schopfer and Plachy, 1973), bean (De Greef et al., 1977), maize (Sharma et al., 1976) and radish (Huault and Klein, 1980). Phytochrome regulation of peroxidase activity is organ specific (Schopfer and Plachy, 1973; Huault and Klein, 1980) and does not

involve the participation of kinetin, gibberellin, acetyl choline, C-AMP or photosynthesis and is age dependent (Sharma et al., 1977, 1979a, 1979b).

Plant hormones have different effects on peroxidase which varies with the tissue and the concentration of the hormone. IAA activates peroxidase in Phaseolus vulgaris (Brunner, 1978), ripening bananas (Desai and Despanday, 1979) and mung bean seedlings (Dendsay and Sacher, 1982); inhibits in pea stem cuttings (Henry and Jorden, 1977) and Phaseolus radiatus seedlings (Ram et al., 1976) but has no effect in wheat aleurone (Tao-Karling and Khan, 1975) and Sinapis alba (Legrand and Dabois, 1978). Synthetic auxins IBA and 2,4-D stimulate peroxidase in Phaseolus vulgaris (Brunner, 1978) and napthalene acetic acid (NAA) in cultured wheat cells (Chowdhary et al., 1980) but 2,4-D inhibits it in Sinapis alba (Legrand and Dubios, 1978) and wheat (Fluckiger, 1977). GA stimulates peroxidase in apple embryos (Nikolaeva and Jankelevich, 1976), cultured wheat cells (Chowdhary et al., 1980) and wheat aleurone (Tao-Karling and Khan, 1975) but decreases it in Phaseolus radiatus seedlings (Ram et al., 1976), ripening banana (Desai and Despandey, 1979) and pea stem sections (Henry and Jorden, 1977). Kinetin stimulates peroxidase in apple embryos (Nikolaeva and Jankelevich, 1976) and Lentil (Dubucq, 1976; Gaspar et al., 1975), inhibits it in ripening banana (Desai and Despandey, 1979) and has no effect in wheat aleurone (Tao-Karling and Khan, 1975).

Etherel stimulates peroxidase in lentil (Dubucq, 1976) and sweet potato roots (Buescher et al., 1975) but has no effect in sweet potato leaves (Birecka et al., 1976) and mung bean cotyledons (Dendsay and Sacher, 1978). Abscissic acid increases peroxidase in ripening banana (Desai and Despandey, 1979) but inhibits it in mung bean cotyledons (Dendsay and Sacher, 1978) and lentil (Gaspar et al., 1975). Along with hormones, peroxidase is also affected by heavy metals (Lee et al., 1976; Nag Pratima et al., 1981), herbicides (Taburi et al., 1981; Decleire et al., 1982) and fungicides (Szymczak and Marian, 1980).

ATPase

There are well characterized ATPases (ATP phosphohydrolase E.C. 3.6.1.8) in all living organisms. They are present in bacteria (Haddock and Jones, 1977), animals (Knowles et al., 1975) and plants (Lai and Thompson, 1971; Leonard and Hotchkiss, 1976). In plants they are present in all organelles (Robert et al., 1979). Among the cell organells there are well characterized ATPases in mitochondria (Grubmeyer and Spencer, 1978) and choloroplasts (Strotmann and Schumann, 1983). Both in mitochondria and chloroplasts the enzyme is associated with the synthesis of ATP; in mitochondria by means of oxidative phosphorylation and in chloroplasts by photophosphorylation (Boyer et al., 1977). ATPases present in membrane structures are mainly associated

with ion absorption and transport (Dupont et al., 1981). Apart from their presence in cell organells ATPase is also present in cytosolic fraction (Fischer and Hodges, 1969).

ATPase activity increases during germination (Ferreria and Stobart, 1975) and during greening of etiolated leaves (Nadler and Wu, 1974). It decreases with increasing age as well as senescence of leaves (Parida and Mishra, 1980) and cotyledon (Elloitt, 1982). In cotyledons when senescence is retarded by detopping the shoot apex, the ATPase activity increases indicating that the decrease of activity can be prevented by inhibiting or reversing the phenomena. Fruit ripening causes an increase in ATPase in both particulate and cytosolic fractions (Ben-Arie and Faust, 1980).

Mineral nutrition has a significant effect on ATPase activity (Kuiper and Kuiper, 1979). It increases with calcium and magnesium deficiency (Matsumoto and Kawasaki, 1981) and saline conditions (Erdei et al., 1980). During chilling stress ATPase activity decreases in all cell organells in tomato (Ling Cheng et al., 1981) but in pine it increases towards the end of the hardening period and decreases to normal during dehardening period (Hellergren et al., 1983).

ATPase activity of cell membrane fractions is generally stimulated by certain divalent cations. The most effective cations are Mg^{++}, Ca^{++} and Mn^{++}. The stimulation by divalent cation depends on the particular membrane fraction examined, the salt status of the tissue and the plant species (Hodges, 1976). ATPase activity is also stimulated by monovalent ions but this occurs only in the presence of divalent cations. Many salts of monovalent ions are capable of stimulating membrane ATPase activity and it was thought that the cations were responsible for this stimulation (Fisher and Hodges, 1969). However, recent reports suggest that the anions are responsible for stimulating ATPase activity of membrane preparations from leaves of limonium (Hill and Hill, 1973), turnip roots (Rungie and Wiskich, 1973) and oat roots (Blake et al., 1974). It appears from these results that both cation sensitive and anion sensitive ATPases are present in plant cell membranes and the measurement of ATPase activity of heterogenous membrane preparations include both of these enzymes. In nearly all investigations individual salts of monovalent ions are as effective in stimulating ATPase activity as are various combinations of salts. However, KCl and NaCl synergistically stimulate the ATPase activity of membrane preparations (Kylin and Gee, 1970).

Several herbicides, pesticides and toxicants inhibit ATPase activity (Riedel and Christensen, 1979; Watson et al., 1980) possibly by blocking -SH groups (Stom and Rogozine, 1976). Heavy metals which generally cause interruptions in energy mobilizing systems also inhibit ATPase activity (Veltrup, 1981).

Plant hormones have been shown to modulate ATPase activity. IAA stimulates ATPase activity from hypocotyls of mung bean (Kasama and Yamaki, 1973) and Pumpkin (Scherer, 1981) and from rice roots (Erdei et al., 1979). Synthetic auxin 2,4-D also stimulates ATPase activity from soy bean hypocotyls (Scherer and Morre, 1978) but inhibits at concentrations which inhibit cell enlargement (Colombo and Ferrari-Bravo, 1982). ATPase activity is also stimulated by gibberellin (Khokhlovq, 1979) kinetin (Elliott, 1982) and etherel (Henry and Richard, 1979) but is inhibited by abscissic acid (Cocucci and Cocucci, 1977).

The foregoing discussion shows that peroxidase and ATPase bound to membranes are involved in several physiological functions in plants. Since polyamines and guanidines have been reported to protect plants against some of these conditions possibly by controlling the membrane integrity and function, the aim of the studies reported in this thesis

was to investigate whether polyamines and guanidines have any control on the membrane bound peroxidase and ATPase activity. Maize scutellum which is associated with the transport of nutrients and undergoes senescence has been selected for the studies.