CHAPTER 7

Biochemical and Physiological Impact of Major Pollutants

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BACKGROUND

The harmful effects of air pollution on various components of vegetation such as forest trees, agricultural crops, ornamental plants, and lichens, are now well recognized. The major pollutants studied in this regard are sulphur dioxide, ozone, oxides of nitrogen, peroxyacetyl nitrate, and fluoride. These pollutants can have a deleterious effect on a variety of biochemical and physiological processes and on structural organization within the cells.

Following an episode it is often assumed that there has been no injury to vegetation unless visible symptoms of phototoxicity have developed. However, this can be misleading. In many controlled environment studies, air pollutants have been shown to reduce the growth and yield before any visible symptoms appeared. It is now commonly believed that injury initially takes place at the biochemical level (interference with photosynthesis, respiration, lipid and protein biosyntheses, etc.), subsequently progressing to the ultrastructural level (disorganization of cellular membranes), and then to the cellular level (cell-wall, mesophyll, and nuclear breakdown). Finally, visible symptoms develop (chlorosis and necrosis of foliar tissues).

Biochemical injury results when the concentration of the pollutant exceeds the capacity of the tissues to detoxify it through their normal metabolism. The subtle and varied nature of the biochemical and physiological effects pro-
duced by air pollutants suggest that reduction in plant growth and yield because of air pollution may be more widespread and serious than is generally suspected.

SULPHUR DIOXIDE

Stomatal Response

Plants absorb sulphur dioxide (SO₂) mainly by gaseous diffusion through the stomata. Some uptake of SO₂ also occurs from moist cuticular surfaces but is of minor significance. The number of stomata and size of aperture play major roles in the uptake of SO₂, as do regulating factors that can affect the turgidity of guard cells, such as humidity, wind velocity, light, and temperature. Thomas & Hill (1935) showed that absorption of SO₂ was correlated with humidity. The presence of SO₂ in the air has been shown to stimulate stomatal opening (Majernik & Mansfield, 1970, 1971; Mansfield & Majernik, 1970), or closing (Menser & Heggestad, 1966), both of which are regulated by relative humidity and the concentrations of SO₂ and CO₂ in the air (Majernik & Mansfield, 1972). Recently it was demonstrated in *Vicia faba* (Broad or Field Bean) that low concentrations of SO₂ stimulated stomatal conductance within 15 minutes of exposure, and that this persisted for several days (V. J. Black & Unsworth, 1980). This may have been due to extensive destruction of epidermal cells adjacent to the stomata (C. R. Black & V. J. Black, 1979a). High concentrations of SO₂, on the other hand, frequently caused severe ultracellular disorganization (C. R. Black & V. J. Black, 1979b).

The different tolerances of plant species to SO₂ under similar biophysical conditions suggest that fine differences in biochemical and physiological mechanisms, operative in different plants, could influence the sensitivity of a particular plant to SO₂. It has been suggested that SO₂ injury depends on the rate of SO₂ absorption (Thomas, 1951; Bressan *et al.*, 1978; Caput *et al.*, 1978). Recently, Furukawa *et al.* (1980a) found highly significant correlations between foliar injury and the amount of SO₂ absorbed; plants that are sensitive to SO₂ absorbed greater amounts of gas than did those which are resistant to it. In contrast, no correlation between sulphur uptake and foliar injury was observed in *Agropyron smithii* (Lauenroth *et al.*, 1979). Resistance of *Picea abies* (Norway Spruce) seedlings to SO₂ when stomata were open was attributed to cellular mechanisms for detoxifying SO₂ (Oku *et al.*, 1980).

The effects of SO₂ on the biochemical and physiological processes directly related to stomatal response have recently been studied in detail. An increased concentration of carbon dioxide (CO₂) induces stomatal closure. Abscisic acid (ABA) is also known to produce a similar response, and has the ability to control CO₂-induced stomatal closure (Raschke, 1975). Plant sensitivity to SO₂ could therefore also be an indication of changes in the ABA
levels of the fumigated foliage. Kondo & Sugahara (1978) measured the effect of 2 ppm SO₂ on the transpiration rates (to some extent a measure of stomatal behaviour) of sensitive and resistant plants. They found a rapid decrease in the transpiration rate of SO₂-resistant plants, and either a gradual decrease or an increase in transpiration rates of sensitive plants, submitted to this level of SO₂. Analysis of the ABA content of the foliage revealed that the resistant plants contained higher levels of ABA than the sensitive ones. When ABA-treated Raphanus sativus (Radish) was exposed to SO₂, the transpiration rate began to drop immediately, thereby making the plant much more resistant to SO₂ than it was originally. Later experiments by Kondo et al. (1980b), involving a number of SO₂-resistant plant species, confirmed the relationship between ABA content and decreased transpiration rate following SO₂ 'fumigation' (i.e. exposure).

Upon diffusion through the stomata, gaseous SO₂ dissolves in water on the moist cellular surfaces to form sulphite (SO₃²⁻), bisulphite (HSO₃⁻), and other ionic 'species' (depending on the pH of the surrounding cellular surfaces); in such transformation, cellular pH would also be influenced by the generation of protons. The influence of these species on the ABA-related stomatal response was studied recently by Kondo et al. (1980b), using epidermal strips of Vicia faba. In the absence of ABA, SO₃²⁻ slightly stimulated stomatal opening, but in the presence of ABA, SO₃²⁻ produced no effect. No additional decrease in aperture size occurred on adding SO₃²⁻ in the presence of ABA concentrations that, alone, reduced aperture size. Changing the pH (between 4 and 7) similarly did not affect aperture size in the absence of ABA, but during its presence the stomatal aperture size was markedly reduced at pH 4.

These results suggested that ABA-related stomatal closure as a result of SO₂ fumigation was an acidic effect on the surface or cytoplasm of guard-cells. Other studies, however, have reported an increase in stomatal opening at low pH (Squire & Mansfield, 1973; Dittrich et al., 1979). Since in these studies the levels of ABA in the SO₂-fumigated plants were not examined before and after the fumigation (in either sensitive or resistant plant species), the role of ABA in regulating stomatal responses during fumigation remains speculative and contradictory. For example, the transpiration rate of Zea mays (Maize) leaves in response to SO₂ fumigation was similar to that of other resistant plant species, yet the ABA content of the leaves was the lowest of all the plant species examined. Even if the cellular content of ABA was a major factor in controlling the absorption of SO₂, the mechanisms involved in the regulation of ABA levels in guard- and other subsidiary cells following SO₂ fumigation remains to be determined.

It appears that stomatal regulation in plants not possessing the ABA-type mechanism occurs by other biochemical and physiological means. Cellular increase of H⁺ (normally brought about by SO₂) could cause leakage of K⁺,
Cl\(^{-}\), and malate (F. A. Smith & Raven, 1979). In K\(^{+}\)-regulated stomatal opening, anions such as malate and Cl\(^{-}\) play important roles (Raschke, 1979). Malate has been shown to be synthesized in the guard-cells by carboxylation of phosphoenol pyruvate (PEP) (Outlaw & Kennedy, 1978). In Zea mays and Spinacia oleracea (Spinach), phosphoenolpyruvate (PEP) carboxylase is inhibited by SO\(_3^{2-}\) (Ziegler, 1973a; Mukerji & Yang, 1974), which in turn reduces malate synthesis and leads to changes in stomatal opening or plant sensitivity towards SO\(_2\). Another biochemical control of stomatal response occurs through metabolic regulation of glycollate content (Zelitch, 1971). It has been shown that SO\(_3\) inhibits glycollate oxidase activity in needles of Pinus banksiana (Khan & Malhotra, 1982a); this enzyme is also inhibited by low concentrations of SO\(_3^{2-}\) (Zelitch, 1957; Khan & Malhotra, 1982a).

**Biochemical Transformations of Absorbed SO\(_2\)**

The phytotoxic effects of SO\(_2\) are greatly influenced by the ability of plant tissues to convert dissolved SO\(_2\) into relatively non-toxic forms. Sulphite and HS\(_2\)O\(_3\)\(^{-}\) are the major chemical species formed upon dissolution of SO\(_2\) in aqueous solutions; their respective concentrations depend on the pH of the medium (Puckett et al., 1973). Both SO\(_3^{2-}\) and HS\(_2\)O\(_3\)\(^{-}\) have been shown to be phytotoxic to many biochemical and physiological processes (Zeigler, 1975; Malhotra & Hocking, 1976). Plants can overcome these phytotoxic effects by converting SO\(_3^{2-}\) and HS\(_2\)O\(_3\)\(^{-}\) to less-toxic forms. Oxidation of SO\(_3^{2-}\) to sulphate (SO\(_4^{2-}\)) in plant cells can occur by both enzymic and non-enzymic mechanisms, and SO\(_4^{2-}\) thus accumulated is considerably less toxic than SO\(_3^{2-}\) (Thomas et al., 1943).

Plants exposed to SO\(_2\) can accumulate sulphur compounds. Accumulation of large amounts of sulphur takes place at low SO\(_2\) concentrations (Guderian, 1977), while at high SO\(_2\) concentrations the accumulation is impaired due to collapse of stomatal regulation. In general, metabolically active young leaves accumulate more sulphur than older leaves (Guderian, 1977); however, Agropyron smithii accumulates less sulphur in young leaves than in older ones on the same plant (Lauenroth et al., 1979).

Experiments with isotopic SO\(_2\) (\(^{35}\)SO\(_2\)) have shown that there are labelled SO\(_3^{2-}\) and SO\(_4^{2-}\) in the treated foliage (Garsed & Read, 1977a, 1977b). Glycine max (Soybean) leaves exposed to \(^{35}\)SO\(_2\) incorporated five times as much radioactivity in light as in the dark (Garsed & Read, 1977a), the major product being SO\(_4^{2-}\) (Garsed & Read, 1977b). Studies on the residence time of SO\(_3^{2-}\) following SO\(_2\) fumigation, showed that Glycine max cultivars resistant to SO\(_2\) converted SO\(_3^{2-}\) more rapidly to SO\(_4^{2-}\) than the sensitive cultivars (J. E. Miller & Xerikos, 1979). It appears, therefore, that the presence of SO\(_3^{2-}\)-oxidizing mechanisms can influence plant resistance to SO\(_2\).

Sulphite has been oxidized in the light by isolated chloroplasts in a reaction
induced by the electron transport system (McCord & Fridovich, 1969; Asada & Kiso, 1973; Libera et al., 1973; Khan & Malhotra, msa). Sulphite-oxidizing activities have also been reported in isolated mitochondria (Tager & Rautanen, 1956; Arrigoni, 1959; Ballantyne, 1977). Recently, Kondo et al. (1980a) separated various $\text{SO}_3^{2-}$-oxidizing activities and characterized their natures. In a number of plants they found a cytochrome $c$-linked $\text{SO}_3^{2-}$-oxidizing substance that had a low molecular weight and was non-proteinaceous in nature. High-molecular-weight $\text{SO}_3^{2-}$-oxidases, on the other hand, were not linked to cytochrome $c$.

Oxidation of $\text{SO}_3^{2-}$ can also be stimulated by cellular enzymes such as peroxidase, cytochrome oxidase, and ferredoxin-NADP reductase, and by catalysts such as metals and ultraviolet light (Hällgren, 1978). Production of the superoxide radical ($\text{O}_2^-$) in the chloroplast during illumination also stimulates $\text{SO}_3^{2-}$ oxidation. This is supported by the observation that, in the presence of superoxide dismutase (SOD), photooxidation of $\text{SO}_3^{2-}$ was inhibited (McCord & Fridovich, 1969; Asada & Kiso, 1973); however, free-radical oxidation of $\text{SO}_3^{2-}$ was accelerated by indoleacetic acid (tryptophan) and Mn$^{2+}$, but not by SOD (Yang & Saleh, 1973). The superoxide radical formed during illumination has been found in vivo (Radmer & Kok, 1976) and in isolated chloroplasts (Asada & Kiso, 1973; Epel & Neumann, 1973; Asada et al., 1974); it could serve as a source of other active oxygens ($^1\text{O}_2$, OH•, and $\text{H}_2\text{O}_2$).

In the presence of $\text{SO}_3^{2-}$ and HS$\text{O}_3^-$, more $\text{O}_2^-$ is formed by free-radical chain oxidation than otherwise. This process also generates OH• and $\text{SO}_3^{2-}$ radicals. Together these oxidizing radicals can affect a number of cellular mechanisms. Apparently in order to arrest uncontrolled production of such free radicals, plants have developed natural scavengers (Asada, 1980) that protect them from injurious effects. For example, K. Tanaka & Sugahara (1980) found that leaves with a high content of superoxide dimutase (SOD) were resistant to $\text{SO}_2$. Similarly, increased peroxidase activity that often results from fumigation of plants with $\text{SO}_2$ (Horsman & Wellburn, 1975; Keller, 1976; Keller et al., 1976; Khan & Malhotra, 1982b) could be the result of either cellular activity to metabolize $\text{H}_2\text{O}_2$ produced by a free radical mechanism or the oxidation of absorbed $\text{SO}_2$. Ascorbic acid, another scavenger of free radicals, has also been reported to protect plants from $\text{SO}_2$ injury (Keller & Schwager, 1977; Grill et al., 1979a).

The $^{35}\text{SO}_2$ absorbed by plant leaves does not remain fixed at the site of absorption but has a substantial degree of mobility (Jensen & Kozlowski, 1975; Garsed & Read, 1977c); such translocations occur from leaves to roots, from old leaves to young leaves, and from roots to surrounding medium. Plants can, therefore, act as sinks for atmospheric $\text{SO}_2$.

Absorbed $\text{SO}_2$ can also be utilized by plants in the reductive sulphur cycle. Ziegler (1975) stated that this reduction seemed to involve reactions similar
to \( \text{SO}_4^{2-} \) reduction. As details of the reduction mechanisms have been well reported elsewhere (Schiff & Hodson, 1973; Hallgren, 1978), only recent work on this topic will be dealt with here. Sulphite reduction in plant leaves occurs mainly in the chloroplasts (Schiff & Hodson, 1973). Recently it has been shown that, in plants with \( \text{C}_4 \)-type photosynthesis, the assimilation of sulphur is initiated only in the bundle-sheath chloroplasts; the mesophyll chloroplasts are inactive (Gerwick et al., 1980).

Sulphur dioxide can specifically inhibit the activity of adenosine phosphosulphate (APS) sulphotransferase in the leaves of *Phaseolus vulgaris* (Kidney Bean) seedlings, but the inhibition can be reversed upon the removal of \( \text{SO}_2 \) (Wyss & Brunold, 1980). This is a key enzyme in the intermediary \( \text{SO}_4^{2-} \)-reduction metabolism, and is involved in \( \text{SO}_4^{2-} \) transfer from APS to a carrier (Car-SH) to form a carrier-S-sulphite complex. Other sulphur compounds (\( \text{SO}_4^{2-}, \text{H}_2\text{S}, \) and cysteine) also affect the activity of this enzyme (Wyss & Brunold, 1979, 1980). It is possible that the high \( \text{SO}_2 \) concentration used in the above studies affected the enzyme owing to excessive accumulation of \( \text{SO}_4^{2-} \) and other sulphur compounds.

Recently it has been suggested that both \( \text{SO}_3^{2-} \) and \( \text{SO}_4^{2-} \) are transported to the inner chloroplast membranes by phosphate translocators (Hampp & Ziegler, 1977) and that light stimulates this process (Ziegler & Hampp, 1977). The light-activated uptake and reduction appear to be controlled by photosystem-dependent electron transport, as inhibition of the photoelectron transport system completely blocked both the uptake and reduction of \( \text{SO}_4^{2-} \) (Ziegler & Hampp, 1977). Sulphate reduction was dependent on ATP produced during photophosphorylation, because uncoupling of photophosphorylation inhibited \( \text{SO}_4^{2-} \) uptake and its reduction. Sulphite, on the other hand, was metabolized directly, without any ATP requirement; in fact \( \text{SO}_4^{2-} \) uptake and reduction were stimulated by the uncoupler (Ziegler & Hampp, 1977).

As \( \text{SO}_3^{2-} \) can be utilized directly in sulphur metabolism, these results are of considerable significance in \( \text{SO}_2 \) metabolism—especially in view of the inhibitory effects of \( \text{SO}_2 \) on chloroplast photoelectron transport and photophosphorylation systems (to be discussed later). In leaves of *Lemna minor* (Lesser Duckweed), however, it has been shown that absorbed \( \text{SO}_2 \) was assimilated only after oxidation to \( \text{SO}_4^{2-} \) (Brunold & Erismann, 1976). In support of direct reduction of \( \text{SO}_3 \), Ziegler (1977a) showed that sulphur from \( \text{SO}_2 \) and \( \text{SO}_3^{2-} \) was incorporated in the chloroplast lamellae to a much greater extent than sulphur from \( \text{SO}_4^{2-} \). She suggested that \( \text{SO}_3^{2-} \) was either directly incorporated into the sulphinic groups of the sulphanilin acids as reported by Benson (1963), or was taken up at the binding sites in the lamellae (Schwenn et al., 1976).

Accumulation of SH-containing amino acids following fumigation with \( \text{SO}_2 \) has been reported for a number of plant species (Ziegler, 1975; Grill et al., 1979b, 1980; Malhotra and Sarkar, 1979); similar accumulations have
also been noticed upon continuous application of SO$_3^{2-}$ to the cultures of *Chlorella vulgaris* (Soldatini et al., 1978). Photoreduction of SO$_2$ to H$_2$S and its subsequent release from plants was reported by Cormis (1968). Similar results were reported with $^{35}$SO$_2$ in *Spinacia oleracea* leaves and isolated chloroplasts (Silvius et al., 1976). Reduction of SO$_2$ to H$_2$S could be catalysed by sulphite reductase, found in many plants (Schiff & Hodson, 1973). It is interesting to note that short-time fumigation of *Spinacia oleracea* plants with SO$_2$ caused a marked increase in free and masked thiol in the chloroplasts (Miszalski & Ziegler, 1979). Such an increase in the concentration of SH compounds can influence the natural balance of sulphydryl (SH)/disulphide (SS) level in the cells. Plants exposed to injurious concentrations of SO$_2$ emit considerable amounts of H$_2$S; a positive correlation between such emissions and SO$_2$ resistance was shown in a recent report by Sekiya et al. (1980).

**Photosynthesis**

The response of photosynthetic processes to SO$_2$ depends on the duration of exposure and the concentration of SO$_2$. Short-time exposure to low SO$_2$ concentrations generally stimulates photosynthesis in a number of plants. In most cases, however, high doses of SO$_2$, or continued exposures at even low concentrations, are very inhibitory to photosynthesis. The sensitivity of plants to SO$_2$ at low concentrations can nevertheless be influenced by environmental factors such as wind-speed, light, and humidity. Ashenden & Mansfield (1977) showed that 0.11 ppm SO$_2$ significantly reduced the growth of *Lolium perenne* (Rye grass) only at high wind-speed; the lack of effect at lower wind-speed was due to increased boundary-layer resistance. In *Vicia faba*, a decrease in net photosynthesis occurred at SO$_2$ concentrations exceeding 0.035 ppm, and the decrease was influenced by boundary-layer resistance and light intensity (V. J. Black & Unsworth, 1979a, 1979b). Hällgren (1978) has reviewed physiological conditions that influence the SO$_2$ effect on the photosynthetic response of intact leaves.

A rapid decrease in net photosynthesis in leaves of *Helianthus annuus* (Sunflower) upon exposure to 1.5 ppm SO$_2$ led Furukawa et al. (1980b) to suggest that the chloroplasts were the primary site of attack. Biochemical reactions associated with photosynthesis have been studied in order to ascertain more specifically the sites and mechanisms of SO$_2$ action. Ziegler (1972) observed that SO$_3^{2-}$ inhibited ribulose bisphosphate (RuBP) carboxylase in *Spinacia oleracea* chloroplasts. The kinetics of SO$_3^{2-}$-inhibition indicated competition between HCO$_3^-$ and SO$_3^{2-}$ at the CO$_2$-binding sites of the enzyme, which suggests that the concentration of CO$_2$ (or HCO$_3^-$) at the site of carboxylation influences the degree of SO$_3^{2-}$ inhibition.

A similar type of competitive inhibition by SO$_3^{2-}$ with respect to HCO$_3^-$ has been observed with isolated preparations of RuBP carboxylase from a
lichens, *Pseudevernia furfuracea* (Ziegler, 1977b). Recently, however, Gezeilus & Hällgren (1980) showed that the \( \text{SO}_3^{2-} \)-inhibition of RuBP carboxylase from *Pinus sylvestris* (Scots Pine) and *Spinacia oleracea* was non-competitive with respect to \( \text{HCO}_3^- \), and the nature of the inhibition was not affected by the presence of \( \text{SO}_3^{2-} \) during the activation. It is interesting to note that the enzyme from *Pinus sylvestris* was inhibited similarly by 10 mM \( \text{SO}_3^{2-} \) or \( \text{SO}_4^{2-} \). As \( \text{SO}_4^{2-} \) was also shown to inhibit *Spinacia oleracea* RuBP carboxylase non-competitively with respect to \( \text{HCO}_3^- \) (Trown, 1965), the possible presence of \( \text{SO}_3^{2-} \)-oxidizing systems in crude enzyme extracts of *Pinus sylvestris* cannot be overruled. Gezeilus & Hallgren (1980) have minimized such an interference by assaying in \( \text{N}_2 \) atmosphere. Using purified preparations of RuBP carboxylase from *Pinus banksiana* (Jack Pine), Khan & Malhotra (1982a) found a competitive type of inhibition by \( \text{SO}_3^{2-} \), with a \( K_i(\text{SO}_3^{2-}) \) value of 5 mM; the enzyme was also inhibited by \( \text{SO}_4^{2-} \), but to a much lesser extent than by \( \text{SO}_3^{2-} \).

It must be pointed out that crude extracts and isolated chloroplasts of *Pinus banksiana* needles contain very active \( \text{SO}_3^{2-} \)-oxidizing systems (Khan & Malhotra, msa). It is therefore necessary that purified preparations of RuDP carboxylase be used for assessing the nature of \( \text{SO}_3^{2-} \) inhibition. Reduction in the activity of RuBP carboxylase was observed in *Alnus crispa* (Green Alder) and *Betula papyrifera* (Paper Birch) seedlings upon fumigation with 0.34 and 0.51 ppm \( \text{SO}_2 \) (Khan & Malhotra, msb). Reduction in the activity of RuBP carboxylase upon exposure to gaseous \( \text{SO}_2 \) has also been reported in leaves of other plants (Horsman & Wellburn, 1975; Miszalski & Ziegler, 1980).

Sulphite also affects phosphoenolpyruvate (PEP) carboxylase (Ziegler, 1973a; Mukerji & Yang, 1974), which is involved in the \( \text{C}_4 \) pathway of photosynthesis. In *Zea mays* (Ziegler, 1973a), the \( \text{SO}_3^{2-} \) inhibition was competitive with respect to \( \text{HCO}_3^- \), being similar to inhibition of RuBP carboxylase; however, the inhibitor constant \( K_i \) of \( \text{SO}_3^{2-} \) for PEP carboxylase, was much higher than for RuBP carboxylase. As PEP carboxylase has much higher affinity for \( \text{CO}_2 \) (Ziegler, 1973a) than has RuBP carboxylase, the replacement of \( \text{CO}_2 \) by \( \text{SO}_3^{2-} \) at the carboxylation site would be more difficult in the PEP carboxylase-catalysed reaction than in the RuBP carboxylase reaction.

The effect of \( \text{SO}_3^{2-} \) on PEP carboxylase from *Spinacia oleracea* (Mukerji & Yang, 1974) appeared to be different from that of the *Zea mays* enzyme. The *Spinacia oleracea* enzyme was stimulated by \( \text{SO}_3^{2-} \) at a low concentration (0.5 mM) and was inhibited at a high concentration (5 mM). Sulphite inhibited the enzyme activity, and the inhibition was of a mixed type with respect to \( \text{HCO}_3^- \). Kinetic analysis of the *Spinacia oleracea* enzyme suggests that \( \text{SO}_3^{2-} \) affected PEP carboxylase activity, not by interfering with the substrate binding sites, but by unspecific binding with the enzyme protein (Mukerji &
Yang, 1974). As $SO_3^{2-}$ has been shown to bind rapidly with PEP in vitro (Lehmann & Benson, 1964), such a reaction during PEP carboxylase assay would limit the availability of PEP for carboxylation. In the presence of PEP carboxylase, however, a $SO_3^{2-}$ reaction with PEP did not appear to occur, because $SO_3^{2-}$ either had no effect or acted non-competitively with respect to PEP (Ziegler, 1973a; Mukerji & Yang, 1974). Bisulphite compounds have also been shown to inhibit PEP carboxylase reaction (Osmond & Avadhani, 1970; Mukerji & Yang, 1974). The extent to which PEP carboxylase activity is influenced by gaseous $SO_2$ has not been determined.

In addition to influencing the carboxylation reactions, $SO_2$ can affect photosynthesis by attacking photosynthetic electron transport and photophosphorylation reactions. Chloroplasts isolated from needles of Pinus contorta (Lodgepole Pine) treated with varying concentrations of aqueous $SO_2$ showed that, at a low concentration (50 ppm), $SO_2$ stimulated Hill (evaluation of oxygen due to photolysis of water and the transfer of electrons to an electron acceptor, e.g. ferricyanide and dyes are generally used as artificial electron acceptors) reaction activity, but this activity was completely inhibited at high concentrations (500–1000 ppm) (Malhotra, 1976). The chloroplasts isolated from old tissues were more sensitive to $SO_2$ than were those from young, actively growing tissues. A decrease in the Hill reaction activity was accompanied by swelling and disintegration of chloroplast membranes. Such alterations in the membranes can cause disorganization of the two photosystems. Photosystems I and II are both localized in the membranes of chloroplasts, and have been separated by density-gradient centrifugation of digitonin-treated chloroplasts (Boardman, 1968).

Recently, Shimazaki & Sugahara (1980a, 1980b) studied in detail the effect of gaseous $SO_2$ on chloroplast photosystems in Spinacea oleracea. Fumigation with $SO_2$ at 1 and 2 ppm for 1 hour produced no effect on 2,6-dichloroindophenol (DCIP) photoreduction (Hill reaction); however, there was rapid inhibition following longer exposures. Shimazaki & Sugahara investigated the site of $SO_2$ attack in the electron transport systems by studying both photosystems.

Electron transport of both the whole chain and Photosystem II was inhibited to the same magnitude by $SO_2$, but $SO_2$ did not inhibit electron-flow from reduced DCIP to nicotinamide adenine dinucleotide phosphate (NADP) under uncoupled conditions, which suggests that the site of $SO_2$ action in the photosystems was associated with Photosystem II and not Photosystem I. A similar effect of $SO_2$ was observed in photosystems of Lactuca sativa (Garden Lettuce) chloroplasts (Shimazaki & Sugahara, 1980b). The work with chloroplasts isolated from $SO_2$-fumigated leaves of Lactuca sativa (Shimazaki & Sugahara, 1980b) demonstrated that the site of $SO_2$ action was located closer to the oxidizing side rather than the reducing side of Photosystem II. This was supported by the observation that the addition of an artificial
electron-donor, diphenylcarbazide (DCP), did not change the rate of DCIP reduction in Photosystem II.

The work of Shimazaki & Sugahara (1980b) also suggests that SO$_2$ did not inactivate the electron-flow from the reductant to the primary electron acceptor (Q) of Photosystem II. Time-course analysis of fluorescence intensity in SO$_2$-treated plants indicated that SO$_2$ inhibited the accumulation of reduced Q. Furthermore, the addition of 3-(3',4’dichlorophenyl)-1,1-dimethyl urea (DCMU), an inhibitor acting on the reducing side of Photosystem II (Bishop, 1958), caused a rapid increase in fluorescence in SO$_2$-inhibited chloroplasts, which suggests that Q was in the oxidized state. This could happen because of SO$_2$-inactivation of either the primary electron donor or the reaction centre itself in the electron transport chain.

The above inhibitory effects of gaseous SO$_2$ on the photosystems are different from those reported from earlier studies in which treatment of isolated chloroplasts with solutions of SO$_3^{2-}$ either produced no overall effect on electron transfer (Asada et al., 1965) or else stimulated a non-cyclic-type of electron transfer (Libera et al., 1973). The effect of gaseous SO$_2$ seems to be specific and not associated with the acidity-related decrease in Photosystem II activity, as a decrease in Photosystem II activity due to low pH could be restored by adding electron donors of Photosystem II but not in chloroplasts from SO$_2$-treated plants (Shimazaki & Sugahara, 1980b). Inhibition of Photosystem II activity by exposure to SO$_2$ was accompanied by a similar inhibition in non-cyclic photophosphorylation, but not in cyclic photophosphorylation (Shimazaki & Sugahara, 1980a). On the other hand, treatment of isolated chloroplasts with solutions of SO$_3^{2-}$, HSO$_3^-$, and SO$_2$, inhibited both cyclic and non-cyclic photophosphorylations (Asada et al., 1965; Libera et al., 1973; Silvius et al., 1975).

The differences between the in vivo effects of SO$_2$ on both photoelectron transport and phosphorylations, and the in vitro effects of treatment of isolated chloroplasts with aqueous SO$_2$ (HCO$_3^-$, SO$_3^{2-}$, and SO$_2$), are difficult to reconcile. Shimazaki & Sugahara (1980a) have attributed such differences to production of O$_2^-$ and other radicals during photooxidation of SO$_3^{2-}$. As SO$_4^{2-}$ is formed by the oxidation of SO$_3^{2-}$, it is possible that the effects observed in vitro are the effects of free radicals and SO$_4^{2-}$. It has been shown that SO$_4^{2-}$ irreversibly inhibits both cyclic and non-cyclic photophosphorylations (Ryrie & Jagendorf, 1971).

**Pigments**

As chlorophyll and other plant pigments are necessary in harnessing light-energy by Photosystems I and II, the effect of SO$_2$ on these pigments would greatly influence the photosynthetic ability of plants. Rao & LeBlanc (1965) found that destruction of chlorophyll occurred in lichens following exposure
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...to large doses (5 ppm for 24 hours) of gaseous SO$_2$. At this high concentration, chlorophyll molecules were degraded to phaeophytin and Mg$^{2+}$. A similar conversion of chlorophyll to phaeophytin can occur with acids or acidic substances. In this process Mg$^{2+}$ in the chlorophyll molecule is replaced by two atoms of hydrogen, thereby changing the light-spectrum characteristic of the chlorophyll molecules.

A decrease in chlorophyll-content has often been suggested as an indicator of air pollution (mainly SO$_2$) injury. In sensitive lichens, chronic exposure to even a low concentration (0.01 ppm) of SO$_2$ resulted in a loss of chlorophyll (Gilbert, 1968). In *Evernia mesomorpha*, control fumigation with a low level of SO$_2$ caused a gradual decline in the chlorophyll content, which was accompanied by a decrease in photosynthesis; the effects on both chlorophyll destruction and photosynthesis became more rapid and pronounced at 0.34 ppm SO$_2$ concentration (Malhotra & Khan, unpublished results).

Sulphur dioxide can influence chlorophyll by various mechanisms. Malhotra (1977) showed that treatment of *Pinus contorta* needles with aqueous SO$_2$ markedly affected the chlorophyll content of the foliage, and that this was not due to increased acidity alone. As, in *vivo*, chlorophyll is stabilized by its organization as a complex with proteins, it is possible that SO$_2$ first attacks this complex before the actual breakdown of chlorophyll occurs. In *Pinus*, low aqueous SO$_2$ concentrations had very little effect on either chlorophyll *a* or *b*, but at high concentration chlorophyll *a* appeared to be much more sensitive to aqueous SO$_2$ than was chlorophyll *b*. Increased destruction of chlorophyll *a* was accompanied by a parallel increase in phaeophytin *a* and loss of Mg$^{2+}$ from the tissues.

As the destruction of chlorophyll in pine needles was markedly greater in the presence of aqueous SO$_2$ than in the presence of HCl solutions of the same pH, it was suggested that SO$_2$ destroyed chlorophyll as a result of its strong redox properties. In needles of *Pinus contorta*, a decline in chlorophyll *b* content in the presence of SO$_2$ did not produce a corresponding increase in phaeophytin *b*, which suggests a different mechanism in chlorophyll *b* breakdown. In fact, an increase in chlorophyllide *b* content following SO$_2$ treatment suggested that chlorophyll *b* breakdown was probably a result of splitting of the phytol chain by chlorophyllase. The maximum increase in tissue chlorophyllide *b* content was observed at 50 ppm aqueous SO$_2$ concentration—the same concentration that produced the maximum stimulation in chlorophyllase activity.

Rapid *in vitro* chlorophyll destruction can also be caused by free radicals produced during the oxidation of HSO$_3^-$-catalysed decomposition of linoleic acid hydroperoxide (Peiser & Yang, 1977, 1978). Recently, Shimazaki *et al.* (1980) presented evidence that SO$_2$ fumigation of leaves increases the formation of O$_2^-$ in chloroplasts that in turn destroys chlorophylls. Superoxide radical has been shown to influence chlorophyll at very low concentrations...
(10^{-8} \text{ to } 10^{-7} \text{ M}) \text{ (Asada et al., 1977). In Spinacia oleracea leaves, gaseous } \text{SO}_2 \text{ destroyed chlorophyll } a \text{ more rapidly than chlorophyll } b, \text{ but the loss of chlorophyll } a \text{ was not accompanied by a corresponding increase in phaeophytin } a \text{ (Shimazaki et al., 1980). As scavengers of free radicals inhibited chlorophyll breakdown in Spinacia oleracea leaves, it was suggested that } \text{SO}_2 \text{ destroys chlorophyll mainly by a free-radical oxidation. This was further supported by the observation that chlorophyll } a \text{ breakdown was inhibited by superoxide dismutase. Sulphur dioxide inhibits the superoxide dismutase activity in the fumigated tissues (Shimazaki et al., 1980). Furthermore, accumulation of malonaldehyde, a lipid peroxidation product, and a decrease in chlorophyll } a \text{ in } \text{SO}_2\text{-fumigated Spinacia oleracea leaves, was related to the free-radical oxidation of chlorophyll.}

Chlorophylls and other pigments in the chloroplasts are stabilized by forming complexes with proteins, but little is known about the effect of SO$_2$ on these complexes. Recently, Sugahara et al. (1980) showed that, in vitro, water-soluble protein complexes of chlorophyll and chlorophyllide were stable and were not destroyed by even 40 mM SO$_{3}^{2-}$. The photoconversion of the dark form of the chlorophyll $a$ and chlorophyllide $a$ protein complex (CP 668) to the illuminated form (CP 743) was, however, inhibited by SO$_{3}^{2-}$. The inhibition was apparently due to irreversible denaturation of the protein component in the pigment protein complex, probably caused by destruction of disulphide bonds.

Respiration

Photorespiration has been found to be inhibited by SO$_2$ (Ziegler, 1975). Exposure of Lolium perenne to SO$_2$ (0.15 ppm) caused an inhibition in glycine and serine synthesis, even though CO$_2$ fixation was stimulated; this suggests inhibition of photorespiration (Koziol & Cowling, 1978). A similar reduction in these amino acids following exposure to SO$_2$ was reported by Tanaka et al. (1972b). Glycollate oxidase, an important enzyme for the synthesis of glycine and serine, was inhibited by low concentrations of SO$_{3}^{2-}$ in vitro (Zelitch, 1957; Paul & Bassham, 1978; Khan & Malhotra, 1982a) and by gaseous SO$_2$ (Khan & Malhotra, 1982a). In leaves of Nicotiana tabacum (Tobacco), however, exposure to a high SO$_2$ concentration (1.3 ppm for 18 hours) induced an increased synthesis of glycollate oxidase (Soldatini & Ziegler, 1979).

It has been suggested that a decrease in photorespiration as a result of SO$_2$ or SO$_{3}^{2-}$ exposure is due to the formation of glyoxylate bisulphite, which is a potent inhibitor of glycollate oxidase (Zelitch, 1957). Glyoxylate bisulphite was found to accumulate in the leaves of Oryza sativa (Rice) plants exposed to high concentrations of SO$_2$ (Tanaka et al., 1972a). Similarly, Pisum sativum (Garden Pea) exposed to high SO$_2$ concentration, produced toxic bisulphite
compounds of glyceraldehyde, α-ketoglutarate, pyruvate, and oxaloacetate (Jiracek et al., 1972). The high concentration of SO\textsubscript{2} used in these studies leaves doubt as to the formation of such compounds in plants exposed to the more common, low levels of SO\textsubscript{2} experienced under field conditions.

Unlike photorespiration, dark respiration either showed no response or was stimulated by SO\textsubscript{2} (V. J. Black & Unsworth, 1979b; Furukawa et al., 1980b). The significance of these results at the biochemical level was not ascertained. The in vitro addition of SO\textsubscript{3}\textsuperscript{2−} to plant mitochondrial preparations, however, inhibited the formation of adenosine triphosphate (ATP) (Ballantyne, 1973). It is possible that, due to its strong reducing nature, SO\textsubscript{3}\textsuperscript{2−} caused reduction of electron transport components and uncoupling, which resulted in subsequent decreased ATP synthesis.

Sulphur dioxide and its dissolved reactive ‘species’ can affect a variety of other cellular metabolites and enzyme systems through either non-specific or specific mechanisms. At high concentrations, SO\textsubscript{3}\textsuperscript{2−} reacts with a number of metabolites (Mudd, 1975a; Petering & Shih, 1975; Malhotra & Hocking, 1976), but at the low concentrations that are generally present under field conditions, such reactions will be of rare occurrence. The following sections discuss the effects of low and medium concentrations of SO\textsubscript{2} on enzymes and cellular metabolites.

Amino Acids and Proteins

In a number of plant species, free amino acid content increases following SO\textsubscript{2} fumigation (Arndt, 1970; Jäger & Grill, 1975; Malhotra & Sarkar, 1979). However, in sulphur-deficient Lolium perenne (Ryegrass), SO\textsubscript{2} fumigation caused a decrease in total amino acids and amines, asparagine, and glutamine (Cowling & Bristow, 1979). In general, SO\textsubscript{2} fumigation results in an increase specifically in sulphur-containing amino acids (Ziegler, 1975).

Increased contents of glycine, alanine, thionine, lysine, and methionine, in needles of Pinus banksiana treated with SO\textsubscript{2}, were thought to be due mainly to increased breakdown of needle proteins (Malhotra & Sarkar, 1979). Further work showed that SO\textsubscript{2} decreased the soluble cytoplasmic and chloroplast protein contents of the needles, and that the decrease was higher in the chloroplast than in the soluble cytoplasmic fraction (Khan & Malhotra, msc). A decrease in the total protein content upon SO\textsubscript{2} fumigation has been reported for a number of plants (Fischer, 1971; Godzik & Linskens, 1974; Constantinidou & Kozlowski, 1979). Such a decrease could be attributed to breakdown of the existing proteins and to reduced de novo synthesis. Exposure of an epiphytic lichen, Evernia mesomorpha, to 0.1 ppm SO\textsubscript{2} for 2 days, resulted in a considerable reduction in protein biosynthesis, and this effect became more severe after longer exposures. Fumigation for 3 days at 0.34
ppm SO₂ caused a very pronounced inhibition that was irreversible in clean air (Malhotra & Khan, ms). Similarly, SO₂ (0.34 ppm) markedly inhibited de novo biosynthesis of both cytoplasmic and chloroplast proteins in Pinus banksiana (Jack Pine) the effect being most marked on the chloroplast proteins (Khan & Malhotra, ms). These results indicate that membrane-associated processes are more sensitive to SO₂ than are the cytoplasmic processes.

In Pisum sativum, SO₂ fumigation at 0.3 ppm concentration for 18 days resulted in a marked increase in free and bound polyamines such as putrescine and supermidine (Priebe et al., 1978). Polyamines, which are metabolic products derived from amino-acids, play important roles in nucleic acid metabolism and in the regulation of cellular pH (Cohen, 1971). The increased formation of polyamines in Pisum sativum leaves upon exposure to SO₂, was accompanied by a marked increase in the contents of arginine and ornithine—the precursors of putrescine and supermidine (Preibe et al., 1978). The arginine content of Pinus banksiana needles also increased upon fumigation with SO₂ (Malhotra & Sarkar, 1979); however, its relevance to polyamine synthesis was not studied. The accumulation of these metabolic products following SO₂ fumigation led Priebe et al. (1978) to suggest that increased polyamine synthesis was a regulatory process for binding excessive H⁺ that was produced in the tissues as a result of SO₂ absorption.

It has been suggested that polyamines, whose basicity is comparable to that of NaOH, could form polyvalent cations by binding H⁺ and thus act as buffering compounds in the cells (Priebe et al., 1978). In seedlings of Hordeum vulgare (Barley), acidic conditions were shown to activate the enzymes arginine decarboxylase and N-carbamylputrescine amidohydrolase, which are involved in the synthesis of putrescine (T. A. Smith & Sinclair, 1967).

Several enzymes involved in amino-acid metabolism have also been shown to be affected by SO₂. In Pisum sativum, fumigation with SO₂ affected glutamate dehydrogenase activity by stimulating reductive amination on one hand and inhibiting oxidative deamination on the other (Pahlich et al., 1972). These effects were attributed to changes in isoenzyme pattern (Pahlich et al., 1972) and to enzyme kinetics (Pahlich, 1971). Fumigation of Pisum sativum seedlings with SO₂ inhibited the mitochondrial glutamate oxaloacetate transaminase; the cytoplasmic enzyme showed no effect (Pahlich, 1973). The inhibition of the mitochondrial enzyme by SO₃²⁻/HSO₃⁻ was of a mixed type. On the other hand, the transaminase(s) activity (glutamate-oxaloacetate and glutamate-pyruvate) of Pisum sativum seedlings was slightly stimulated by 0.2 to 2 ppm SO₂ fumigation (Horsman & Wellburn, 1975). Rabe & Kreeb (1980), however, showed that transaminases (alanine and aspartate) were stimulated only by low SO₂ concentrations; at high concentrations they were inhibited.
**Biochemical and Physiological Impact of Major Pollutants**

**Lipids and Fatty Acids**

Lipids are important constituents of biological membranes. In chloroplasts, glycerolipids constitute about 50% by weight of thylakoid membranes (James & Nichols, 1966). A major portion of these glycerolipids is present as monogalactosyl diglyceride, digalactosyl diglyceride, and sulphoquinovosyl diglyceride. In *Pinus contorta*, SO$_2$ caused a marked reduction in the concentration and composition of these glycolipids (Khan & Malhotra, 1977) in both young and fully developed needles. As galactolipids have been shown to be involved in the structure and function of chloroplasts (Shaw et al., 1976), it was suggested that structural alterations in *Pinus contorta* chloroplasts (Malhotra, 1976) were due to changes in galactolipid concentration and composition (Khan & Malhotra, 1977). Isolated chloroplast preparations of *Pinus banksiana* needles contained enzyme systems responsible for the biosynthesis of these membrane lipids (Khan & Malhotra, 1978).

Lipid biosynthesis in the epiphytic lichen *Evernia mesomorpha* has been shown to be affected by SO$_2$ (Malhotra & Khan, ms). Inhibition caused by low-level SO$_2$ exposures (0.1 ppm) was followed by complete recovery 8 days after termination of fumigation. At 0.34 ppm SO$_2$, biosynthesis was inhibited even after 1 day of fumigation, and on day 3 this inhibition became very severe; there was very little recovery in biosynthetic activity upon removal of SO$_2$.

Decreases in the lipid content following SO$_2$ exposure may be brought about by either reduced synthesis, increased lipase activity, peroxidation of fatty-acid chains, or a combination of the above. Malhotra & Khan (1978) showed that exposure of *Pinus banksiana* and *P. contorta* seedlings to SO$_2$ caused a marked inhibition in the *de novo* synthesis of phospho-, glyco-, and neutral lipids. The magnitude of inhibition was dependent on the concentration and duration of exposure to SO$_2$. The effect was, however, transitory in nature, as the inhibition was partially or completely reversed upon removal of plants from the SO$_2$ atmosphere. As no accumulation of free fatty acids occurred upon SO$_2$ treatment, it was suggested that SO$_2$ did not cause stimulation of lipolytic activity but inhibited the synthetic and acylation processes.

Treatment of *Pinus banksiana* needles with SO$_2$ produced a marked reduction in the content of linolenic acid and an increase in the content of palmitic acid; the effect was more pronounced in young needles than in fully-developed ones (Khan & Malhotra, 1977). As SO$_2$ treatment in both types of needles caused reduction in linolenic acid content and an increase in palmitic acid content, it is suggested that SO$_2$ inhibited both the elongation and desaturation processes. The decline in the linolenic acid content may have occurred by SO$_2$-stimulated free-radical peroxidation reactions, indicated by SO$_2$-related malonaldehyde accumulation (Khan & Malhotra, 1977). The
formation of malonaldehyde also occurs in leaves of other plants injured by SO₂ (Peiser & Yang, 1979; Shimazaki et al., 1980).

Carbohydrates and Organic Acids

Plants exposed to SO₂ exhibit increasing amounts of soluble sugars (Khan & Malhotra, 1977; Koziol & Jordan, 1978; Malhotra & Sarkar, 1979). In *Pinus banksiana*, SO₂ fumigation (0.34 and 0.51 ppm) increased the content of the reducing sugars and reduced that of the non-reducing sugars (Malhotra & Sarkar, 1979). It was suggested that the increase was due to a breakdown of polysaccharides rich in reducing sugars. Koziol & Jordan (1978) showed that SO₂ exposure of *Phaseolus vulgaris* seedlings caused a reduction in starch content. Reduction in non-structural total carbohydrates has also been reported following SO₂ exposure of *Ulmus americana* (American Elm) seedlings (Constantinidou & Kozlowski, 1979). Recently, Bucher-Wallin et al. (1979) found significant increases in the activities of several glycosidases in the foliage of clonal forest trees following exposure to low levels of SO₂, which suggests that the enzymes were involved in the breakdown of polysaccharides. As polyhydric sugars are known to act as scavengers of free radicals (Asada, 1980), this could act as a mechanism to help to cope with increasing SO₂ pollution.

Sulphur dioxide also affects a number of enzymes and metabolites involved in organic acid metabolism. Organic acids act as intermediates of a number of metabolic end-products and help to maintain cellular pH. Any changes in their metabolism would therefore have an influence on plant growth and yield. In *Pinus banksiana* needles, SO₂ decreased the content of two major organic acids, namely quinic acid and shikimic acid, and increased the content of syringic acid, a minor component (Sarkar & Malhotra, 1979). Reduction of ¹⁴C incorporation into organic acids following fumigation of *Pinus thunbergii* (Japanese Black Pine) and *P. densiflora* (Japanese Red Pine) with SO₂ has also been reported (Ishizaki & Hasegawa, 1974). Such changes may be brought about by modification of either the structure or the kinetics of the enzymes involved.

In *Spinacia oleracea*, SO₃²⁻ inhibited NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (Ziegler et al., 1976); however, fumigation with SO₂ (0.7 ppm for 1 hour) caused a significant stimulation of the light-activated enzyme. The stimulation was thought to be due to generation of increased SH groups during fumigation (Miszalski & Ziegler, 1979). Low concentrations of SO₂ similarly stimulated the activities of glucose-6-phosphate and isocitrate dehydrogenases, but high SO₂ concentrations appreciably inhibited the two enzymes (Rabe & Kreeb, 1980). A considerable decline has also been reported in nicotinamide adenine dinucleotide (NAD)-dependent malate dehydrogenase activity of *Pinus banksiana* seedlings
exposed to 0.34 ppm SO₂; isoenzyme analysis showed one less isoenzyme in the SO₂-treated plants compared with the control (Sarker & Malhotra, 1979). Decreases in NAD and NADP-dependent malate dehydrogenase activity by SO₂²⁻ were shown to be due to the SO₃²⁻ effect on the kinetics and multimeric forms of the enzymes (Ziegler, 1974).

**OZONE**

**Stomatal Response**

Stomatal control could be responsible for the differential resistances to ozone (O₃) by seedlings (Treshow, 1970). Humidity, which is highly conducive to increase in stomatal opening, engendered considerable ozone injury to plants (Otto & Daines, 1969) though little or no such injury occurred when stomata were closed (Heath, 1975). In Phaseolus vulgaris, however, maximum sensitivity to O₃ was not related to stomatal number or their resistance to the gas at either surface of the leaf (Evans & Ting, 1974a).

Abscisic acid is known to effect stomatal responses, and its application reduces O₃ injury to plants (Fletcher et al., 1972; Jeong et al., 1980). Fumigation with O₃ increased ABA content in Oryza sativa; the plants resistant to O₃ had higher ABA content than the sensitive ones (Jeong et al., 1980).

**Absorption and Permeability Effects**

Moist surfaces in the mesophyll tissues provide the media in which gaseous O₃ can dissolve; however, little is known about O₃ diffusion and its chemical transformation within the tissues. It has been suggested that O₃ diffuses passively, due to a concentration gradient similar to that of CO₂ (Heath, 1975). The concentration of O₃ in the plant tissues is affected by its solubility, rate of decomposition, and the pH at the site of absorption.

Similarly to SO₂, O₃ can also give rise to the superoxide radical (O₂⁻), which can produce other radicals such as OH⁺, O₂, and H₂O₂. These radicals can oxidize various cellular metabolites (Asada, 1980). A number of membrane constituents such as SH groups, amino acids, proteins, and unsaturated fatty acids, are affected by O₃ (Heath, 1975), probably as a result of free-radical attack.

Permeability changes in O₃-fumigated tissues are thought to be due to leaky plasmalemma; these include changes in permeability to water (Evans & Ting, 1973), glucose (Dugger & Palmer, 1969; Perchorowicz & Ting, 1974), and ions (Evans & Ting, 1974b; Heath & Frederick, 1979). Permeability of mitochondrial (T. T. Lee, 1968) and chloroplast membranes (Nobel & Wang, 1973) is also influenced by O₃. Sutton & Ting (1977) demonstrated that O₃-induced membrane injury can be repaired by energy-dependent processes.
Photosynthesis

Fumigation of plants with \( \text{O}_3 \) inhibited photosynthesis, though this was reversed about 24 hours after the termination of exposure (Hill & Littlefield, 1969; Pell & Brennan, 1973). The direct effect of \( \text{O}_3 \) on carboxylases involved in photosynthetic \( \text{CO}_2 \) fixations are not well known. Thomson et al. (1966) speculated that the granulation of chloroplast stroma, seen in electron micrographs of \( \text{O}_3 \)-treated tissues, was due to oxidation of SH groups in the Fraction 1 protein (RuBP carboxylase). In \textit{Oryza sativa} (Rice) plants, fumigation with \( \text{O}_3 \) reduced the activity of RuBP carboxylase in both young and old leaves 12–14 hours after fumigation, but 48 hours after fumigation the enzyme activity had recovered to some extent only in the young leaves (Nakamura & Saka, 1978).

Treatment of isolated \textit{Spinacia oleracea} chloroplast suspensions with \( \text{O}_3 \) inhibited electron transport in both photosystems; Photosystem I, however, was more sensitive than Photosystem II (Coulson & Heath, 1974). The treatment inhibited photophosphorylation by reducing electron transport and not by uncoupling. Chang & Heggestad (1974) have also demonstrated the effect of \( \text{O}_3 \) on Photosystem II in \textit{Spinacia oleracea} chloroplasts. Inhibition by \( \text{O}_3 \) of Photosystem II was proportional to the extent of visible injury in relatively resistant (Bel-B) and sensitive (Bel-W,) \textit{Nicotiana tabacum} (Tobacco) leaves (Rhoads & Brennan, 1978). Ozone treatment of isolated chloroplasts from the two varieties, however, inhibited their electron transport activities equally, which suggests that either the \( \text{O}_3 \) effect was at sites other than the electron transport systems or the relatively resistant variety was more capable of repairign the injury than the sensitive one.

Schreiber et al. (1978) showed that \( \text{O}_3 \) (0.3 ppm) altered the fluorescence characteristics in \textit{Phaseolus vulgaris} long before any visible symptoms of injury appeared. Their data also suggested that a low concentration of \( \text{O}_3 \) for a long exposure was more injurious than a high concentration for a short exposure. On the basis of specific change in fluorescence, they suggested that the site of \( \text{O}_3 \) action was within the photosynthetic apparatus. The initial damage was thought to be at the donor site of Photosystem II (\( \text{H}_2\text{O}\)-splitting enzyme system) and not directly on its reaction centre. Increasing \( \text{O}_3 \) exposures also resulted in the inhibition of electron transport from Photosystem II to Photosystem I.

In addition to influencing the activity of the electron transport system, \( \text{O}_3 \) affected the chlorophyll content of the treated plants (Leffler & Cherry, 1974). In \textit{Phaseolus vulgaris}, a high correlation was found between chlorophyll loss and visible necrosis (Knudson et al., 1977), and the ratio of Chlorophyll \( a \) to Chlorophyll \( b \) declined with increasing \( \text{O}_3 \) injury. Such changes were due either to more \( \text{O}_3 \) susceptibility of chlorophyll \( a \) than chlorophyll \( b \), or to their altered biosynthesis. Beckerson & Hofstra (1979c) observed a
decrease in chlorophyll \(a\) and \(b\) following exposure of *Phaseolus vulgaris* to \(O_3\).

**Respiration**

The effect of \(O_3\) on respiration is variable; it can either stimulate (Todd, 1958; Dugger & Palmer, 1969; Barnes, 1972) or inhibit (MacDowell, 1965) plant respiration. Ozone exposure inhibited respiration and phosphorylation in *Nicotiana tabacum* leaf mitochondria (T. T. Lee, 1967). In *Phaseolus vulgaris*, there was no effect on respiration immediately after \(O_3\) exposure, but significant stimulation occurred within 24 hours. The content of ATP and total adenylate, however, increased immediately following \(O_3\) exposure (Pell & Brennan, 1973).

The effect of \(O_3\) on photorespiration and related metabolic processes is not known at the present time.

**Amino Acids and Protein Metabolism**

Ozone can cause either an increase or a decrease in amino-acid content of plants (Ting & Mukerji, 1971; Tomlinson & Rich, 1967; Tingey *et al.*, 1973). Using \(^{14}\text{CO}_2\), Wilkinson & Barnes (1973) demonstrated an increase in the synthesis of alanine and serine after exposure of *Pinus strobus* (White Pine) and *P. taeda* (Loblolly Pine) to \(O_3\). Similarly, the protein content of *Glycine max* leaves increased 24 hours after exposure to \(O_3\) (Tingey *et al.*, 1973), which suggests an increase in the biosynthesis of amino acids and proteins. On the other hand, the protein content of other plants was markedly reduced by \(O_3\) treatment (Ting & Mukerji, 1971; Constantinidou & Kozlowski, 1979), thereby suggesting either a breakdown of the existing proteins or no synthesis of the new ones.

Chang (1971) reported that in *Phaseolus vulgaris* leaves, \(O_3\) can cause dissociation of the chloroplast polysomes but not of the cytoplasmic polysomes. It has been suggested that such changes were induced by desiccation brought about by \(O_3\) (Heath, 1975).

Ozone can also affect nitrogen metabolism by inhibiting nitrate reductase in *Glycine max* leaves; the inhibition of the enzyme, however, occurred only under *in vivo* and not *in vitro* conditions (Tingey *et al.*, 1973). This was explained as inhibition in the *in vivo* formation of reduced NADP (required for the enzyme activity) and not as a direct effect on the enzyme. In *Zea mays* (Maize) and *Glycine max* leaves, \(O_3\) treatment inhibited the activities of both nitrite and nitrate reductases (Leffler & Cherry, 1974). Nitrite reductase, a chloroplastic enzyme, was inhibited more severely by \(O_3\) than was the cytoplasmic nitrate reductase.
Fatty Acid and Lipid Metabolism

Treshow et al. (1969) reported that exposure to O₃ resulted in a loss of pigmentation and neutral lipids in the fungus Colletotrichum lindemuthianum, though its fatty acid composition remained unaffected. The response in the lipids was attributed to an inhibitory effect on their biosynthesis, probably brought about as a result of SH oxidation. On the other hand, recent work with two sensitive cultivars of Nicotiana tabacum showed that fumigations of plants with O₃ (0.25 or 0.30 ppm for 6 hours) significantly increased the lipid content of the leaves but caused a decrease in triglyceride fatty acids (Trevathan et al., 1979). It was speculated that the increase in lipids was an injury response, because rust infection causes a similar accumulation of lipids (Lösel, 1978). Variable effects of O₃ have been reported on fatty acid content in different plant species (Tomlinson & Rich, 1969, 1970, 1971; Swanson et al., 1973). In Nicotiana tabacum leaves, O₃ caused a reduction in all fatty acids, the largest decreases being in palmitic (16 : 0) followed by linolenic (18 : 3) acids (Tomlinson & Rich, 1969). Such a reduction could occur by inhibition of fatty acid synthesizing and desaturating of enzymes.

Ozone can also affect polyunsaturated fatty acids by oxidative mechanisms. Such oxidations can change the properties of the membranes. Malonaldehyde, an oxidation product of unsaturated fatty acids, is formed upon treatment of unsaturated lipids with O₃ (Mudd et al., 1971b); similarly, increase in malonaldehyde was accompanied by a decrease in unsaturated fatty acid (Frederick & Heath, 1970). Increased production of malonaldehyde has also been observed in leaves of Oryza sativa (Nakamura & Saka, 1978) and Phaseolus vulgaris (Tomlinson & Rich, 1970) after exposure to O₃.

The direct effect of O₃ on the lipid biosynthesis of isolated chloroplasts has been reported by Mudd et al. (1971a, 1971b). Ozone impaired the ability of chloroplasts to metabolize [1-¹⁴C] acetate or acetyl-CoA in lipid biosynthesis. This was attributed to oxidation of essential SH groups (Mudd et al., 1971a). The biosynthesis of galactolipids in chloroplasts was similarly inhibited by O₃ and by other agents that bind SH groups (Mudd et al., 1971b). Ozone treatment, however, did not change the fatty acid composition, which led Mudd et al. (1971a) to suggest that fatty acids of the chloroplast membranes were not very accessible to oxidation by O₃.

Ozone-treated plants also exhibit a decrease in free sterols (Tomlinson & Rich, 1971, 1973; Spotts et al., 1975; Menser et al., 1977; Trevathan et al., 1979) and an increase in sterol glycosides and esterified sterol glycosides (Tomlinson & Rich, 1971). These changes may affect membrane permeability.

Carbohydrates and Related Metabolism

Ozone can either increase (Tingey et al., 1973, 1976a) or decrease (P. R. Miller et al., 1969; Constantinidou & Kozlowski, 1979) the level of soluble
sugars and carbohydrates in the leaves of treated plants. Tingey et al. (1973) showed that in *Glycine max* leaves, a single acute exposure of $O_3$ (0.49 ppm) caused a significant initial decrease of reducing sugars, which was followed by a subsequent increase. The starch content did not change, however—which suggests that the initial decrease in soluble sugars could have resulted from a depression in the photosynthetic rate, and that the subsequent increase could be due to reduced sugar utilization and/or reduced sugar translocation for carbohydrate synthesis. In *Pinus ponderosa* (Ponderosa Pine), $O_3$ exposure resulted in an increase in the content of soluble sugars, starch, and phenols, in the foliage, and a decrease in their contents in the roots (Tingey et al., 1976b).

Using $^{14}$CO$_2$, Wilkinson & Barnes (1973) demonstrated that, in *Pinus strobus* and *Pinus taeda*, $O_3$ decreased the incorporation of the label into simple soluble sugars while increasing its incorporation into sugar phosphates. Such alterations in the carbohydrate metabolism could be attributed to the effect of $O_3$ on enzyme activities. A decrease in the activity of glyceraldehyde-3-phosphate dehydrogenase and an increase in glucose-6-phosphate dehydrogenase in *Glycine max* leaves was attributed to $O_3$-induced inhibition of the glycolytic pathway and stimulation of the Pentose Phosphate Pathway (Tingey et al., 1975, 1976a). Stimulation of the Pentose Phosphate Pathway also occurs in diseased and aged plant tissues (Goodman et al., 1967; Gibbs, 1966).

As sugars also act as scavengers of free radicals (Asada, 1980), it is possible that an increase in their levels can partly overcome the phytotoxic effects of free radicals produced by $O_3$. This is a tentative speculation and awaits experimental evidence.

**Phenols and Related Metabolism**

Keen & Taylor (1975) found an accumulation of isoflavonoids in $O_3$-treated plants and suggested that plants under stress can trigger such metabolic responses. In *Medicago sativa* (Alfalfa or Lucerne), an accumulation of 4', 7-dihydroxyflavone occurred after exposure to 0.2 ppm $O_3$ for 2.5 hours, and its concentration markedly increased with increasing symptoms of visible injury (Hurwitz et al., 1979). Increased production of isoflavonoids can also occur in response to pathogenic infections (Keen & Kennedy, 1974).

Enzymes involved in phenol metabolism, such as phenylalanine ammonia lipase, polyphenol oxidase, and peroxidase, are stimulated by $O_3$ (Tingey et al., 1976a). Activation of these enzymes would stimulate oxidation of phenols to quinones and cause accumulation of their polymerization products. These products could be responsible for a necrotic appearance of injured leaves. Peroxidase activity has been reported to increase in plants after exposure to $O_3$ (Curtis & Howell, 1971; Dass & Weaver, 1972; Tingey et al., 1975, 1976a; Curtis et al., 1976). Isoenzyme analysis of peroxidase in two cultivars of *Glycine max* (Soybean) with varying sensitivities to $O_3$ showed that, in the
resistant cultivar, O\textsubscript{3} caused an increase in the activity of a few isoenzymes whereas in the sensitive cultivar it increased the activity of all isoenzymes (Curtis \textit{et al.}, 1976).

Plants subjected to stress produce elevated levels of ethylene (Abeles, 1973), which was also found in plants exposed to O\textsubscript{3} (Craker, 1971). Ethylene is also a product of normal plant metabolism and has been shown to stimulate the activities of phenylalanine ammonia lyase, polyphenol oxidase, and peroxidase (Abeles, 1973). It is therefore uncertain whether the changes in the activities of these enzymes occur as a direct effect of O\textsubscript{3}-induced ethylene production.

\textbf{OXIDES OF NITROGEN}

\textit{Stomatal Response}

Little is known about the effects of oxides of nitrogen (\textit{NO}_x) on plant stomata and their biochemistry. Because of greater absorption by plants of labelled nitrogen dioxide (\textsuperscript{15}NO\textsubscript{2}) during the day than at night, it was suggested that the gaseous uptake was dependent on stomatal aperture (Yoneyama \textit{et al.}, 1979; Kaji \textit{et al.}, 1980). High stomatal resistance to NO\textsubscript{2} absorption in the dark was considered to be the reason for decreased NO\textsubscript{2} uptake in \textit{Phaseolus vulgaris}. NO\textsubscript{2} inhibited transpiration in illuminated leaves, apparently through causing partial stomatal closure (Srivastava \textit{et al.}, 1975).

\textit{Absorption and Biochemical Transformation}

Formation of nitrate (\textit{NO}_3\textsuperscript{-}) and nitrite (\textit{NO}_2\textsuperscript{-}) has been demonstrated in plants fumigated with \textit{NO}_2\textsuperscript{-}; initially both \textit{NO}_3\textsuperscript{-} and \textit{NO}_2\textsuperscript{-} are formed in equal amounts, and this is followed by accumulation of only \textit{NO}_2\textsuperscript{-} (Zeevaart, 1976). Nitrite is more toxic than \textit{NO}_3\textsuperscript{-} (Mudd, 1973), and in many plants it is detoxified by enzymic mechanisms up to a certain concentration.

Plants absorb gaseous NO\textsubscript{2} more rapidly than NO (Bennett \& Hill, 1975), mainly because NO\textsubscript{2} reacts rapidly with water, while NO is almost insoluble. Fumigation experiments using labelled NO\textsubscript{2} (\textsuperscript{15}NO\textsubscript{2}) further demonstrated that absorbed NO\textsubscript{2} gas is easily converted to \textit{NO}_3\textsuperscript{-} and \textit{NO}_2\textsuperscript{-} before further utilization in plant metabolism (Yoneyama \& Sasakawa, 1979; Kaji \textit{et al.}, 1980). The NO\textsubscript{2} injury to plants occurs either as a result of acidification or because of a photooxidation process (Zeevaart, 1976).

\textit{Photosynthesis}

Decreased photosynthesis has been demonstrated upon exposure of plants to
gaseous NO and NO$_2$, even at concentrations that do not produce visible injury (Hill & Bennett, 1970; Bennett & Hill, 1975; Capron & Mansfield, 1976). The combined effect of the two gases was additive; the NO effect, however, was much more rapid than the effect of NO$_2$ (Hill & Bennett, 1970). Fumigated plants can recover their photosynthetic ability upon transfer to clean air (Hill & Bennett, 1970; Bennett & Hill, 1975). Srivastava et al. (1975) showed that a decrease in photosynthesis in *Phaseolus vulgaris* was related to NO$_2$ concentration and length of exposure.

Exposure of *Pisum sativum* to NO$_2$ produced little change in RuBP carboxylase activity (Horsman & Wellburn, 1975). Exposure of *Pinus banksiana* and *Picea glauca* (White Spruce) to low levels of NO$_2$ (0.2 to 1 ppm for 2 weeks), however, increased RuBP carboxylase activity; the extent of increase was dependent on NO$_2$ concentration (Khan & Malhotra, ms). Short exposures (48 hours) of *Pinus banksiana* to a relatively high concentration of 2 ppm NO$_2$ also stimulated the activity of RuBP carboxylase as well as that of glycollate oxidase; the stimulation was, however, greater in a solution of glycollate oxidase (174%) than in one of RuBP carboxylase (112%). As glycollate oxidase is involved in photorespiration, an increase in its activity to greater than that of RuBP carboxylase would imply a drop in photosynthesis. In contrast, the activities of both RuBP carboxylase and glycollate oxidase were markedly inhibited in *Betula papyrifera* and *Alnus crispa*, even at a low NO$_2$ concentration (0.2 ppm) (Khan & Malhotra, ms). Photosynthetic inhibition brought about by NO$_x$ could be explained as competition for NADPH between the processes of nitrite reduction and carbon assimilation in chloroplasts. The acidity produced by NO$_2$ could also influence electron-flow and photophosphorylation. As photoelectron systems are associated with chloroplast membranes, any changes in their structures would influence activities of the photosystems. Nitrogen dioxide has been shown to cause swelling of chloroplast membranes (Wellburn et al., 1972). Biochemical and membrane injury may result if ammonia, produced from NO$_2$, is not rapidly incorporated into amino-forms; ammonia has been shown to inhibit photosynthesis by uncoupling electron transport (Avron, 1960) and by inducing structural alterations (Puritch & Barker, 1967).

Little is known concerning NO$_x$ effect on plant pigments involved in photosynthesis. Nash (1976) showed that NO$_2$ fumigation caused a significant reduction in the chlorophyll content of various lichen species. Unlike lichens, fumigation of *Pisum sativum* with NO$_2$ resulted in an increase (10%) in the chlorophyll content (Horsman & Wellburn, 1975). Zeevaart (1976) suggested, on the basis of visible injury, that NO$_2$ strongly inhibited pigment synthesis in the developing leaves; as development of chlorosis was generally light-dependent, it is possible that photooxidative processes may have affected the pigments.
**Amino Acids and Proteins**

NO\(_2\) has been shown to stimulate amino acid synthesis from \(^{14}\)CO\(_2\) (Matsushima, 1972). Plants have the ability to metabolize the dissolved NO\(_x\) through their NO\(_3^-\) assimilation pathway:

\[
\text{NO}_x \rightarrow \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+ \rightarrow \text{amino acids} \rightarrow \text{proteins}
\]

Plants exposed to gaseous NO\(_2\) can accumulate and metabolize various products of the above pathway (Zeevaart, 1976; Yoneyama & Sasakawa, 1979; Kaji et al., 1980). Induction of nitrate reductase is known to occur by NO\(_3^-\) (Bevers & Hageman, 1969); and upon fumigation with NO\(_2\) (Zeevaart, 1974). Stimulation of NO\(_3^-\) reductase in fumigated plants causes increased production of NO\(_2^-\), which upon subsequent reduction can be utilized for amino acid synthesis. Reduction of NO\(_3^-\) and NO\(_2^-\) to NH\(_4^+\) was greatly stimulated in light, which suggests that the process of photosynthesis was the major supplier of the reductant (Zeevaart, 1976).

Isolated chloroplasts with a high rate of CO\(_2\) fixation contain an enzyme system that is capable of reducing NO\(_2^-\) in the presence of light (Magalhaes et al., 1974; Miflin, 1974). The addition of ferredoxin stimulated such a reduction (Bevers & Hageman, 1969). A functional association of Photosystem I and ferredoxin with NO\(_2^-\)-reduction in the chloroplasts has also been demonstrated (Neyra & Hageman, 1974). Plaut et al. (1977) showed that NO\(_2^-\) reduction in *Spinacia oleracea* chloroplasts was more rapid in the presence of HCO\(_3^-\) than in its absence, provided that the NO\(_2^-\) was added several minutes after the HCO\(_3^-\). This led them to suggest that CO\(_2\)-fixation products may have a regulatory role in NO\(_2^-\) reduction.

Using labelled NO\(_2\) (\(^{15}\)NO\(_2\)), it has been shown that most of the absorbed NO\(_2\) was transformed into reduced organic nitrogen compounds; the incorporation of \(^{15}\)N was predominantly in the amide nitrogen of glutamine, followed by glutamate, aspartate, alanine, and amino-butyric acid (Yoneyama & Sasakawa, 1979; Kaji et al., 1980). It was thought that, following NO\(_2^-\) reduction, the NH\(_4^+\) was metabolized by glutamine synthetase (GS) and glutamate synthetase (GOGAT) systems. Differences in the distribution of \(^{15}\)N occurred, depending on whether plants were fumigated during the day or at night (Kaji et al., 1980). At night the label was present mainly in the amide nitrogen, with a small amount in amino acids, while during the day the amino acids synthesis was greatly stimulated. This led to the speculation that light stimulates GOGAT and not GS (Ito et al., 1978; Kaji et al., 1980).

In the manner of NO\(_2\), NO also affected nitrogen metabolizing enzymes. For example, Wellburn et al. (1976) showed that NO fumigation caused an appreciable increase in the activity of nitrite reductase. Later work (Wellburn et al., 1980) showed that NO not only stimulates nitrite reductase but also affects other related enzymes. Fumigation of a Tomato (*Lycopersicum*
esculentum) cultivar (Ailsa Craig) that is sensitive to NO, resulted in a significant increase in the activities of glutamate dehydrogenase (GD), glutamate pyruvate transaminase, and glutamate oxaloacetate transaminase (GOT); fumigation of an NO-resistant cultivar (Santo) stimulated only GOT. These results suggest that nitrogen from NO$_x$ could be assimilated by plants through different metabolic systems (GS and GOGAT or GD).

**Fatty Acids and Lipids**

In *Chlorella pyrenoidosa*, NO$_2^-$ (25 mM) markedly inhibited lipid biosynthesis; the inhibition was greater in the dark than in the light (Yung & Mudd, 1966). It is possible that this was due to an increased supply of NADPH in the light-stimulated reduction of NO$_2^-$ to NH$_4^+$, which does not affect lipid biosynthesis even in the dark (Yung & Mudd, 1966). Little is known about the effect of NO$_x$ in lipid metabolism in vascular plants. Fumigation of *Pinus banksiana* seedlings with 2 ppm NO$_2$ for 48 hours inhibited the biosynthesis of lipids characteristic of chloroplast membranes (phospholipids and galactolipids) long before any visible symptoms of injury appeared (Khan & Malhotra, msd).

Oxides of nitrogen have the potential to oxidize unsaturated fatty acids. It is, however, not yet known what NO$_x$ concentration in the atmosphere would affect these fatty acids in plant membranes.

**Other Metabolic Processes**

Dark respiration in primary leaves of *Phaseolus vulgaris* was more severely inhibited by increasing doses of NO$_2$ than was photosynthesis, and the inhibition was not reversed quickly upon removal of NO$_2$ from the atmosphere (Srivastava et al., 1975).

Peroxidase activity was found to be stimulated by fumigation with NO$_2$. This fumigation of *Pinus banksiana* at 2 ppm for 48 hours stimulated peroxidase activity by 25%, while in *Alnus crispa* the enzyme was stimulated (35%) at a much lower NO$_2$ dose (0.2 ppm for 24 hours) (Khan & Malhotra, msb). No visible symptoms of injury were detected in either *Pinus banksiana* or *Alnus crispa* following these fumigations. Stimulation in peroxidase activity also occurred in plants fumigated with NO (Wellburn et al., 1976).

**PEROXYACYL NITRATES**

Peroxyacetyl nitrate (PAN) is the most commonly studied homologue of peroxyacyl nitrates, and is a very phytotoxic component of photooxidative smog.

As with other gaseous pollutants, PAN is absorbed mainly through the
stomata. In plant leaves, its inhibitory effect on photosynthesis is dependent on light (Taylor et al., 1961; Dugger et al., 1963).

Recent work has shown that fumigation conditions can influence the response of photosynthesis in lichens (Sigal & Taylor, 1979). Long exposures at low concentrations of PAN reduced photosynthesis in species such as Parmelia sulcata and Hypogymnia enteromorpha, but had no effect on Collema nigrescens. Short fumigations at high concentrations, on the other hand, produced a slight photosynthetic stimulation in all the species tried except Hypogymnia enteromorpha. Dugger et al. (1965), showed that fumigation at a low concentration of PAN for a short duration inhibited O₂ evolution in chloroplasts of treated plants, but had no effect on photophosphorylation. Treatment of isolated chloroplasts with PAN, however, inhibited electron transport, photophosphorylation, and CO₂-fixation. Coulson & Heath (1975) also showed that exposure of isolated Spinacia oleracea chloroplasts to PAN inhibited electron transport in both photosystems.

The inhibitory effect of PAN on enzymes has been attributed to its ability to oxidize SH groups in proteins and metabolites such as cysteine, reduced glutathione, CoA, lipoic acid, and methionine (Mudd, 1975b). The oxidizing nature of PAN is not limited to SH groups, because it can also oxidize NADH and NADPH, which would eventually interfere with metabolic reactions involving these reduced coenzymes. For more detailed accounts of biochemical effects of PAN, the reader is referred to reviews by Ziegler (1973b) and Mudd (1975b).

**FLUORIDES**

**Uptake and Stomatal Response**

Fluorides in the air occur in either gaseous or particulate form. The gaseous form (HF) is absorbed through the leaves, while the particulate form is generally adsorbed on the outer surfaces of the plant and is thus less injurious to plants (Davison & Blakemore, 1976). The accumulation of fluoride and development of injury in plants exposed to airborne fluorides depend on both environmental and biological factors. Gaseous fluoride absorption through stomata can influence stomatal responses. Poovaiah & Wiebe (1973) demonstrated a partial closure of Glycine max stomata after 1 hour of exposure to 0.15 ppm HF; the effect, however, became more severe after 4 hours of exposure. Stomatal opening did not recover completely in clean air until the following day. HF fumigation of Glycine max also severely depressed the transpiration rate and caused water-absorption potential and leaf temperature to increase. Plant uptake of fluoride was greater during daytime fumigations than at night. To date, nothing is known about the biochemical basis of such responses.
The site of fluoride accumulation inside the leaf cells is not known with certainty, although it has been suggested that, after passing through the cell-wall, fluoride attacks cytoplasmic membranes and is partially retained there and perhaps transferred to the vacuoles (Treshow, 1971). Chloroplasts have also been suggested as the site of fluoride accumulation in leaves of *Citrus sinensis* (Chang & Thompson, 1966).

**Photosynthesis**

Exposure of *Gladiolus* leaves to HF caused a decline in photosynthetic CO$_2$-fixation, but the effect was transient (Thomas & Hendricks, 1956). Little is known about the effect of fluoride on the enzymes involved in photosynthetic CO$_2$-fixation.

At concentrations that did not produce visible injury, HF had no effect on the activity of PEP carboxylase in leaves of *Phaseolus vulgaris* (McCune *et al.*, 1964). Yang & Miller (1963b), on the other hand, reported an increase in the activity of PEP carboxylase and dark CO$_2$-fixation in fluoride-injured leaves of *Glycine max*.

Potassium fluoride has been shown to inhibit the Hill reaction activity of *Phaseolus vulgaris* chloroplasts (Ballantyne, 1972). Ballantyne *et al.* (1979) also found, however, that application of fluoride through *Phaseolus vulgaris* petioles had little effect on Hill reaction activity of the leaf chloroplasts. Further work is needed to determine the site of fluoride action in these two cases.

The mechanism by which fluorides affect chlorophyll in plant leaves is not clear. Low concentrations of fluoride (1.3 to 12.4 ppb) caused slight depressions in the amounts of chlorophyll $a$ and chlorophyll $b$; however, the chlorophylls returned to their normal levels after a recovery period (Weinstein, 1961). It has been suggested that fluoride causes a reduction in chlorophyll synthesis (McNulty & Newman, 1961).

**Respiration**

Fluorides can stimulate or inhibit plant respiration (Treshow, 1971; Chang, 1975). J. E. Miller & G. W. Miller (1974) observed that, depending on the duration of exposure, HF caused either a stimulation or a depression in intact tissue respiration. Respiratory rates and adenosine triphosphatase (ATPase) activity from isolated mitochondria showed the same pattern as intact tissue respiration, but mitochondrial phosphorylation was severely inhibited regardless of inhibition or stimulation of tissue respiration. Fluoride also stimulated mitochondrial swelling and caused leakage of proteins, which suggests that membranes were the primary site of fluoride action.

Psenak *et al.* (1977) showed that KF inhibited succinate oxidation more
severely than it inhibited malate and NADH oxidations in mitochondria of *Brassica oleracea* (Cabbage) and *Glycine max*. The difference was suggested as being due to membrane association of succinoxidase and matrix location of malate oxidase. Respiration changes in fluoride-treated plants could also be attributed to changes in the activities of the oxidative enzymes (C. Lee et al., 1966).

**Amino Acid and Protein Metabolism**

The amino acid metabolism is markedly affected in plants exposed to fluoride. In leaves of *Glycine max* fumigated with HF, necrosis was accompanied by an increase in free amino acids and asparagine content (Yang & Miller, 1963a). Marked increases in free amino acids and amines (asparagine/glutamine) were also reported in needles of *Picea abies* (Norway Spruce) at all stages of HF-induced injury (Jäger & Grill, 1975). Among the amino acids analysed, HF reduced the content of only alanine—perhaps due to inhibition of enolase activity. It is, however, not certain whether the observed increase in free amino acids was due to increased breakdown of cellular proteins or to inhibition of protein synthesis, or to both.

Chang (1970a, 1970b) has shown that NaF caused a decrease in the size of both free and bound ribosomes, as well as a decrease in their protein and RNA content. The disorganization of the ribosomal systems was suggested to be due to an increase in the activity of ribonuclease (RNAase) upon fluoride treatment.

**Fatty Acid and Lipid Metabolism**

Not much is known about the effect of fluoride on plant fatty acid and lipid metabolism. Yee-Meiler (1975) observed a significant increase in the activity of non-specific esterase in foliage of *Betula verrucosa* (Silver Birch) and *Picea abies* plants placed at various distances from a fluoride emission source. Increased esterase activity may have an influence on the metabolism of lipids, including those involved in plant membranes.

Recently, Simola & Koskimies-Soininen (1980) demonstrated the effect of KF on the fatty acid composition of lipid fractions from *Sphagnum fimbriatum* gametophytes; the effect was more pronounced in gametophytes grown at 25°C than at 15°C. Fluoride caused an increase in palmitic acid (16 : 0) and a decrease in linoleic acid (18 : 2) in all lipid fractions, while linolenic acid (18 : 3) decreased in glyco- and neutral lipids. The authors suggested that fluoride inhibited elongation of the fatty acids; however, their results showed that KF also caused an increase in stearic (18 : 0) and oleic (18 : 1) acids in a number of lipid fractions from material grown at 25°C. It therefore appears that inhibition of chain elongation was not the only event
affected by KF, and that a decrease in $18:2$ and $18:3$ fatty acids may also be due to inhibition of the desaturase(s) activity. Such changes in the composition of membrane lipids could influence metabolic functions associated with cellular membranes.

**Carbohydrate and Organic Acid Metabolism**

Plants fumigated with HF show changes in sugars, polysaccharides, and organic acid contents (Adams & Emerson, 1961; Weinstein, 1961; Yang & Miller, 1963a; McCune et al., 1964). Some changes in the carbohydrates and organic acids can be explained as the direct effects of fluorides on various enzymes involved in the metabolism. Enzymes such as glucose-6-phosphate dehydrogenase (G. W. Miller, 1958; C. Lee et al., 1966), enolase (McCune et al., 1964; C. Lee et al., 1966), and phosphoglucomutase (Ordin & Altman, 1965), have been shown to be affected by fluoride.

Psenak et al. (1977) tested the effects of various fluoride compounds on the activity of three isolated malate dehydrogenase isoenzymes and found that NaF and KF had no effect on the enzyme activity although these compounds inhibited malate oxidation in the mitochondria. Among other fluoride compounds, fluoropyruvic acid also inhibited isolated enzymes. It is possible that, in contrast to isolated enzymes, the sensitivity in the cells and organelles was influenced by other aspects of the metabolism.

**Other Metabolic Functions**

McCune et al. (1970) observed that HF had no effect on either the level of acid-soluble nucleotides or the distribution of $^{32}$P into various nucleotide fractions from leaves of treated plants. Similarly, HF fumigation had no effect on acid-soluble phosphorus compounds (Pack & Wilson, 1967). Yee-Meiler (1975) studied the effects of various levels of fluoride on the activity of plant acid phosphatase, and found no correlation between the enzyme activity and the extent of plant exposure to fluoride. The acid phosphatase activity in *Pinus banksiana*, however, was severely inhibited by fluoride (Malhotra & Khan, 1980). The effects of fluoride on the activity of acid and alkaline phosphatases have been summarized by Chang (1975).

Keller & Schwager (1971) reported an increase in peroxidase activity of several forest tree species with decreasing distances from a fluoride emission source; the increase in peroxidase activity was related to fluoride content of the tissues. The investigators suggested that this increase was a direct response to high fluoride uptake by the foliage, rather than an artifact of fluoride dust interaction with the enzyme during extraction. This reasoning was based on the fact that *in vitro* addition of NaF and CaF$_2$ had no effect on the enzyme. Increased peroxidase activity is thought to be an indication of
premature ageing, which could have an effect on the growth and yield of vegetation.

**POLLUTANT MIXTURES**

*Stomatal Response*

Very little is known about how stomatal regulation and associated biochemical and physiological processes are influenced by mixtures of pollutants. The results of some recent studies suggest that stomatal physiology may be affected differently by pollutant mixtures as compared with a single pollutant. Thus in *Phaseolus vulgaris* (Kidney Bean) the transpiration rates were stimulated by SO₂ and NO₂ individually, but were inhibited by a mixture of the two (Ashenden, 1979). Synergistic effects on stomatal closure and visible injury in *Helianthus annuus* (Sunflower) have been reported in response to an O₃–NO₂ mixture (Omasa et al., 1980).

In a number of agricultural plant species, stomatal resistance increased more in response to an SO₂–O₃ mixture than to those pollutants singly (Beckerson & Hofstra, 1979a, 1979b). In spite of the similarities in stomatal response, these species exhibited considerable differences in the development of symptoms of visible injury, which suggests that the sensitivity was not dependent on only the stomatal behaviour. Changes in stomatal responses would, however, influence the absorption of each pollutant from the pollutant mixtures. Elkiey & Ormrod (1980) showed that in *Petunia hybrida*, the amount of SO₂ and O₃ absorbed from a mixture was generally less than that from a single pollutant. Similarly, SO₂ uptake in *Pinus banksiana* and *Betula papyrifera* was less in plants treated with a mixture of SO₂ and NO₂ than in those exposed to SO₂ alone (Khan & Malhotra, msb).

*Membrane Permeability*

Membrane permeability has been shown to be affected by pollutants such as SO₂ and O₃ (see earlier sections). Beckerson & Hofstra (1980), using a number of different plant species, demonstrated that the effect of an SO₂–O₃ mixture on membrane permeability is different from that of the same pollutants individually.

In *Phaseolus vulgaris* and *Glycine max*, solute leakage increased significantly after O₃ treatment but not after an O₃–SO₂ mixture treatment, which suggests an antagonistic effect of SO₂ on membrane permeability. On the other hand, in *Cucumis sativus* (Cucumber) and *Raphanus sativus* (Radish), membrane permeability increased more after treatment with an O₃–SO₂ mixture than with O₃ alone.
Photosynthesis and Other Metabolic Processes

White et al. (1974) reported that, in Medicago sativa (Alfalfa or Lucerne), photosynthesis was inhibited synergistically at a low concentration of an $\text{SO}_2$–$\text{NO}_2$ mixture ($0.15 \text{ ppm} + 0.25 \text{ ppm}$, respectively), but not at a high concentration ($0.5 \text{ ppm}$ for both pollutants). Additive effects of an $\text{SO}_2$–$\text{NO}_2$ mixture have been reported on the reduction of photosynthesis in Pisum sativum (Bull & Mansfield, 1974).

Under field conditions, where emissions of gaseous pollutants are often accompanied by high levels of $\text{CO}_2$, the effect of a pollutant mixture on photosynthesis could be influenced by the $\text{CO}_2$ concentration in the atmosphere. Hou et al. (1977) observed that, at a high $\text{CO}_2$ concentration, photosynthesis in Medicago sativa was much less inhibited by a mixture of $\text{SO}_2$ and $\text{NO}_2$ than at a low $\text{CO}_2$ concentration. Other environmental conditions can also modify the photosynthetic response to pollutant mixtures; for example, plants exposed to a mixture of $\text{SO}_2$ and $\text{O}_3$ showed synergistic reduction in photosynthesis, but the effect was greater at low light intensities and high humidity than at high light intensities and low humidity (Carlson, 1979).

Information about the effects of pollutant mixtures on activities of various enzymes is very limited. Horsman & Wellburn (1975) studied the effects of $\text{SO}_2$ and $\text{NO}_2$ mixtures on various enzymes in Pisum sativum, and observed synergistic effects on the activities of RuBP carboxylase and peroxidase. Recent work has also shown that various enzyme activities in several forest tree species exposed to $\text{SO}_2$ and $\text{NO}_2$ mixtures were affected differently from those exposed to single pollutants (Khan & Malhotra, msb). These effects were additive (peroxidase and glycollate oxidase in Pinus banksiana and RuDP carboxylase in Alnus crispa), synergistic (peroxidase and glycollate oxidase in Alnus crispa), and antagonistic (peroxidase and glycollate oxidase in Betula papyrifera).

In Ulmus americana (American Elm), a mixture of $\text{SO}_2$ and $\text{O}_3$ had a different effect on cellular metabolites compared with the effects of these pollutants individually (Constantinidou & Kozlowski, 1979). These effects were either antagonistic, synergistic, or additive, depending upon the type of tissue examined and the length of time after fumigation. Antagonistic effects have also been reported on the destruction of chlorophylls upon treatment with a mixture of $\text{SO}_2$ and $\text{O}_3$ (Beckerson & Hofstra, 1979c). It is therefore obvious that many mixed pollutants can either prevent or accelerate the toxic responses produced by individual pollutants.

**Summary**

Most plants evolve in a predominantly gaseous environment. When the composition of this environment exceeds the critical limits of adaptation and
tolerance, stress is imposed and the most sensitive components of the system begin to malfunction. Any gas can cause such stress when a threshold concentration is reached. Regardless of the air pollutant, the impact invariably involves interactions with one or more biochemical metabolic processes. First exposed are the stomata and their guard-cells, which may respond first if they are sufficiently sensitive. The gas then passes into the intercellular spaces to become dissolved on the moist internal surfaces, characteristically contacting and influencing membranes and the cellular pH. Penetrating the cytoplasmic membrane, a pollutant is relatively free to attack the organelles within and the substances throughout. A pollutant may react with any number of metabolites along its course of migration through the cell. Consequently, numerous reaction-sites may be, and often are, involved.

The reactions affected depend on the properties and chemical form of the pollutant, but certain sites and reactions seem especially prone to disruption: these include membranes whose permeability may be altered; photosynthetic reactions, e.g. photophosphorylation and carboxylation; electron transport and respiration. Metabolic pools are affected: carbohydrates, organic acids and amino acids, proteins and lipids—are all involved, but the specifics vary with the pollutant.

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