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MECHANISM OF ACTION OF AIR POLLUTANTS ON PLANTS

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ABSTRACT

Air pollutants enter the plant cells through open stomata. After crossing the boundaries of cell wall and cell membrane, they react with the chemical constituents of the cytoplasm. Many effects of air pollutants on plants, such as retardation of growth and increased permeabilities of the cell wall and the membrane may be explained on the basis of their chemical reactions with these components. Most of the visible or 'hidden' physiological effects, however, are brought about the changes in some important metabolite of the cell. Changes in the levels of proteins, pigments and nucleic acids following exposure to air pollutants have been recorded. The enzymes are particularly sensitive to air pollutants both *in vivo* and *in vitro*. The effects on enzymes vary from stimulation to inhibition according to the nature and the concentration of the pollutant used and to the system. Changes in enzyme activities may affect plant metabolism more than the change in any other molecule.

THE effects of different air pollutants on plants have been demonstrated by many investigators. Most of them, however, have limited their efforts to study the visible effects or to specifications of environmental factors which induce or reduce the injury[1,2]. In many cases, there is no visible injury but the over all productivity of the plant is reduced due to some metabolic disorders. The mechanism of phytotoxicity, whether visible or hidden, has not been examined in detail for most of the air pollutants, although the types of physiological and biochemical changes following exposure to the pollutants have been reviewed[3-7]. The objective of the present article is to examine the initial reaction(s) of a plant cell in response to the pollutant, which might be leading to an altered physiology or to a visible effect.

AIR POLLUTANTS AND THE STOMATAL MOVEMENTS

Gases and vapours enter leaves and plants through open stomata. The cuticle is also permeable to some extent to gaseous exchanges but the diffusion through cuticle is generally low. Thus, the entry of gaseous pollutants into the plant and their physiological effects on CO_2 and O_2 exchanges and other processes largely

depend upon the opening and closing of stomata. Open and functional stomata were found to be necessary for the damage to occur by air pollutants in the leaves of alfalfa[8], and *Poa annua*[9,10]. Katz[11] also reported damage to sensitive plants by SO_2 under conditions which favoured open stomata. Ozone damage in tobacco leaves was also positively correlated with the stomatal opening and sugar content of the guard cells[12]. Prefumigation darkness, water stress and spray of phenyl–mercuric acetate, the factors which induce stomatal closure protected tomato plants from ozone damage[13]. The abcissic acid which tend to close stomata[14] protected bean leaves against ozone injury[15]. Damage to bean plants with smog on the other hand, occurred irrespective of the stomatal opening[16]. In another instance also, significant reduction in the growth of tomato plants occurred at low light intensity[17] which might have induced partial stomatal closure. The pollutants in these cases might have entered the plant cells either through the cuticle or through stomata often kept permanently open by dust particles jammed in pores[18].

Air pollutants may induce stomatal closure or opening also. Smog causes stomatal closure in tomato leaves[19]. Stomatal closure by ozone in tolerant varieties of maize and pinto bean leaves[20] and several other plants[21] have also been observed. Although closing of stomata may prevent further entry of the pollutant and avoid injury, it may lead to the inhibition of rates of photosynthesis, respiration and transpiration. In bean leaves, however, inhibition of photosynthesis and respiration occurred in various environmental conditions without appreciable decrease in transpiration[22-24]. In an analysis of the different resistances of gaseous exchange, it was concluded that the effect of NO_2 was more pronounced on mesophyll resistance than on stomatal[23]. For example. an exposure of 3·0 ppm NO_2 to bean leaves increased . CO_2 from 9·54 s cm^{-1} to 16·74 at a light intensity of $5{\cdot}25 \times 10^3$ ergs cm^{-2} s^{-1} while rs CO_2 increased from 1·21 to 1·34 only under similar conditions. Sij and Swanson[25] also reported that SO_2 inhibits photosynthesis in pinto bean and maize leaves without causing stomatal closure. Inhibition of photosynthesis in oats, on the other hand, was the result of stomatal closure[21]. Mansfield and Majerink[18] and Bisdoe *et al.*[26] reported that SO_2 causes stomatal opening in the mature leaves of *Vicia faba*. Stimulation of stomatal opening may increase the access of gas to the mesophyll and thus the damage caused will be greater. Further the opening of stomata may cause water stress by increasing transpiration. Some of the symptoms of SO_2 injury closely resemble those caused by high rates of transpiration[27].

Little is known about the mechanism of opening and closing of stomata by the pollutants. In ozone resistant onions, it has been postulated that the membrane of guard cells lose their premeability and leak out the solutes, thereby closing the stomata. The stomata reopen shortly after the O_3 supply is stopped[28].

Membrane Permeability

After their entry through the open stomata, the air pollutants may affect the organisation of cell walls and cell membranes. Single cell systems have been used to study the effect of O_3 on cell permeabilities. Changes in permeability due to O_3 have been observed in *E. coli*[29], yeast[30], *Chlorella*[31] and red blood cells[32]. The leakage of K from chlorella cells was very rapid and occurred between 5 and 15 s after exposure to O_3 and thus the effect on cell membrane was rather primary[31]. Dugger *et al.*[33] have shown that exposure of plants to O_3 changes the permeability of leaf tissues to exogenous substrates and also to the catabolic utilisation of substrates. This may indicate that the air pollutants change the permeability of cell membranes as well as the transloiation of substrates inside the cell. Disintegration of membranes of parenchymatous cells was shown by Thompson *et al.*[34] Studies with isolated cell organelles showed that the permeability of the membranes of organelles is also affected by the pollutants. Fumigation of bean, tobacco, beet, maize, barley and rye plants with O_3 caused the accumulation of amino acids, γ–amino butyric acid and analine and the loss of glutamic acid[35]. Since the enzyme, glutamate decarboxylase, catalysing the conversion of glutamate to amino butyric acid occurs in cytoplasm and glutamate is produced in mi ochondria and chloroplasts, it appears that O_3 increases permeability of the cell organelles in which the glutamate is produced. Changes in organid acids, amides, free sugars and nucleic acid phosphorus following exposure to O_3 may also be associated with the change in membrane permeability of cell organelles[36]. Increase in the permeability of mitochondrial membrane after exposure to O_3 was observed by Lee[37] and Ting and Dugger[38]. Disruption of chloroplast membrane by SO_2 and NO_2 was observed in bean by Wellburn *et al.*[39] Ozone also induced changes in the permeability of outer chloroplast membranes of intact plastids[40]. Enzymes of photosynthetic fixation of CO_2 are associated with the chloroplasts. Hence, inhibition of photosynthesis by oxidants such as NO_2 is attributed to the disruption of chloroplast membranes[23,24].

Air pollutants may affect membrane permeability either by destroying their integrity or by inhibiting their biogenesis. The –SH groups of proteins are susceptible to oxidation by many pollutants. The inacti-

vation of papain by peroxyacetyl nitrate (PAN) was proportional to the amount of free–SH group at the time of exposure[41,42]. Fumigation with O_3 lowered the –SH content of spinach and tobacco leaves[43]. It was further shown by these workers that tobacco and bean leaves treated with –SH binding reagents such as α–iodoadetamide developed symptoms similar to those of O_3 injury. In another investigation, tobacco leaves exposed to O_3 lost 50% of their saturated fatty acids but only 10% of the unsaturated fatty acids[44], suggesting thereby that O_3 affects membrane permeability by inhibiting fatty acid synthesis rather than by destroying them. Mudd and Lugger[45] also found that PAN inhibits synthesis of fatty acids from acetate in spinach chloroplasts. Incorporation of acetate into citrate and glutamate, however, was not inhibited. Treatment of pine needles with aqueous SO_2 produced marked changes in the concentration and composition of glycolipids[46].

Cell membranes play an important role in the cellular metabolism. Disruption in their structure and function will induce several changes inside the cell. For example, it has been suggested that inhibition of CO_2 fixation by bisulphite compounds is through interference with the transport system in chloroplast membrane[47].

Cell Wall Metabolism

It has been observed by several investigators that exposure to F, PAN and O_3 inhibits growth of *Avena* coleoptiles. For the growth of a plant body, biosynthesis and degradation of cell wall is essential. Works of Ordin and his associates demonstrate the inhibition of cellulose and cell wall formation by air pollutants in oat coleoptile tissues[48-51]. Synthesis of cellulose from glucose phosphate involves the enzyme phosphoglucomutase which converts glucose 6–phosphate to glucose 1–phosphate. This enzyme is believed to be the rate limiting one in the process. Inhibition of *in vivo* activity of this enzyme by F, PAN and O_3 was observed by Ordin and Altman[52]. In oat coleoptiles, either GDP–glucose or UDP–glucose units may be utilised in the final reaction of cellulose synthesis. Peroxy acetyl nitrate treatment inactivated UDP–glucose utilising system in oats [50]. Inhibition of this system by O_3, however, was less; although the inhibition of coleoptile growth by O_3 was also as drastic as by PAN. It is possible that O_3 inhibits cell wall synthesis and coleoptile griwth by modifying phosphoglucomutase activity or some other reaction.

Macromolecular Synthesis

Besides the inhibition of cellulose synthesis, air pollutants may affect metabolism of may other macromolecules also. A disbalance between synthesis and degradation of important macromolecules such as nucleic acids, proteins, chlorophyll etc. may induce several changes both visible and invisible. All the changes at morphological, histological or physiological levels may be correlated to the change at molecular level.

One of the most common observations during exposure to air pollutants is flecking or discolouration of leaves. Bleaching during SO_2 exposure has been observed in lichens[53], bryophytes[54,55] and some angiosperms[55]. Treating pine needles with aqueous SO_2 also accelerated chlorophyll degradation[56]. Gilbert[57] studied the time course of degradation of chlorophyll at very low SO_2 concentration (0·01 ppm) in susceptible and resistant species of lichens and bryophytes. Chlorophyll content in susceptible species, *Ramalina farinacea* and *Hypnum cuvissiforme* declined gradually over a period of 40 days, while it was almost similar to control in resistant species, *Lecanora conizaeoides* and *Torula muralis*. Puckett *et al.*[55] observed that the loss of chlorophyll in lichens was due to their oxidation. Depigmentation has been observel with other air pollutants also. Destruction of chlorophyll by F in susceptible species *Quercus patrea* was more than in resistant species *Alnus glutinosa*[58]. Further, chlorophyll *b* was relatively more stable than chlorophyll *a*. Inhibition of pigment synthesis was observed with O_3 also[59]. It also modified the ratio of chlorophyll to β–carotene in the chloroplasts. On an average the control plants had 88·8 μg of β–carotene per 4·8 mg of chlorophyll, while in ozone treated chloroplasts the amount of β–carotene for the same amount of chlorophyll was 54·1 μg[59]. Decrease in carotenoids may make chlorophyll more susceptible to photooxidation.

Although the reduction in chlorophyll may inhibit the rate of photosynthesis, in many cases, inhibition of photosynthesis is rather rapid[21-23,25] and it is unaccompanied by any detectable chlorophyll degradation[60,61]. In bean leaves also a 3·0 ppm NO_2 for 5 h caused discolouration in plants grown at low nitrogen only and that also only after 2 to 4 days of fumigation[24]. Inhibition of photosynthesis, however, was observed at all nitrogen levels and the effect was instant.

Effect of air pollutants on enzyme activities have been investigated. The effects vary from stimulation to inhibition to no effect (Table I). The nature of the effect depends upon several factors; the species of the plant and the nature of the pollutant being the more important ones. Tingey *et al.*[62] exposed Dare and Hood cultivars of soybean to O_3 for 2 h and then measured the activities of glucose 6-phosphate dehydrogenase, phenylalanine ammonia lyase, polyphenol oxidase and peroxidase in the first trifoliate leaves. The

TABLE I

Effects of air pollutants on enzyme activities

Air pollutant	Enzyme	Plant material	Effect	Reference
Sulfur dioxide	RuDP Carboxylase	Pea	Slight increase	71
	Peroxidase	Pea	Slight increase	71
	Peroxidase	Pine needle	Decrease	72
	Glutamate-pyruvate transaminase	Pea	No effect	71
	Glutamate-pyruvate transaminase	various plants	Decrease	73
	Glutamate-oxalacetate transaminase	Various plants	Decrease	73
	,,	Pea	No effect	71
	,,	Pea	Decrease	74
	Glutamate dehydrogenase	Pea	Increase	75, 76
	,,	Various plants	Increase	73
	Chlorophyllase	Pine needles	Increase at 10–50 ppm and decrease at 100 ppm	72
	Catalase	Pine needles	Decrease	77
	Nitrogenase	*Anabaena cylindrica*	Decrease	61
Nitrogen dioxide	RuDP carboxylase	Pea	No effect	71
	Peroxidase	Pea	No effect	71
	Glutamate-pyruvate transaminase	Pea	No effect	71
Nitrogen dioxide	Glutamate-oxalacetate transaminase	Pea	No effect	71
Nitric oxide	Nitrite reductase	Tomato	Increase	78
Peroxy acetyl nitrate	Polysaccharide synthetase	Oat coleoptile	Decrease	50
	Phosphoglucomutase	Oat coleoptile	Decrease	52
	Ribonuclease	Enzyme prep.	No effect	41
	Papain	Enzyme prep.	Decrease	41
Ozone	6-phosphogluconate dehydrogenase	Soybean	Increase	79
	Glucose 6-phosphate dehydrogenase	Soybean	Increase	62, 80
	Phenylalanine ammonia lyase	Soybean	Increase	62, 80
	Polyphenol oxidase	Soybean	Increase	62, 80
	Peroxidase	Soybean	Increase	62, 80–82
	,,	Many plants	Decrease	83
	Catalase	Many plants	Slight decrease	83
	,,	Soybean	No effect	80
	Papain	,,	Decrease	83
	Urease	,,	Slight decrease	83
	Phosphoglucomutase	Bean	No effect	52
	Ribonuclease	Aqueous sol.	Decrease	42
	,,	Bean	Increase	84

TABLE I—*Contd.*

Air pollutant	Enzyme	Plant material	Effect	Reference
Ozone	Glyceraldehyde 3-phosphate dehydrogenase	Soybean	Increase	80
	Ribonuclease	Soybean	No effect	80
	Protease	Soybean	No effect	80
	Acid phosphatase	Soybean	No effect	80
	Esterase	Soybean	No effect	80
	Nitrate reductase	Soybean	Increase	85
Fluoride	Phosphoglucomutase	Oat coleoptile	Decrease	50
	Enolase	Bean	Increase	86
	,,	Maize	Increase	86
	Catalase	Bean	Increase	86
	PEP carboxylase	Bean	No effect	86
	Pyruvate kinase	Maize	Increase	86

activity of all these enzymes increased. But the increase in sensitive cultivar (Dare) was several hours earlier than in the tolerant one (Hood). Change in enzyme activity may affect the metabolism of a plant more than the change in any other molecule. Decrease in protein content following exposure to O_3 has been observed in tobacco mitochondria[37] and in *Lemna minor*[63]. The effect on proteins appears to be more pronounced on *in vitro* system than on intact plant materials[41,64]. These experiments, however, do not demonstrate whether the decrease is due to inhibited synthesis or to accelerated degradation. Godzik and Linskens[65] demonstrated that SO_2 inhibits protein synthesis in bean leaves. An attempt to reveal the mechanism of protein synthesis inhibition by air pollutants was made by Chang[66]. He observed that O_3 break down the polysomes and monosomes into subunits. Further, the chloroplastic ribosomes were more susceptible than cytoplasmic ones. This was probably because chloroplastic ribosomes have more –SH groups and have better protein synthesizing capacity.

Air pollutants react with nucleic acids and nucleotides also. The content of ribosomal RNA in the chloroplastic ribosomes of bean was decreased by O_3[67]. Protein and RNA content of detached tobacco leaves was also inhibited by an exposure to 30 pphm O_3 for 2 h. However, the effect of O_3 on these components in attached leaves was negligible[13]. In another instance, fumigation of pea, lettuce and maize seedlings induced the formation of pyrimidine sulfonic acid[68].

Inhibition of protein synthesis or acceleration of its degradation may enhance senescence in mature leaves which has been observed with O_3[13,69] and aqueous H_2S[70]. In the latter case, H_2S enhanced degradation of chlorophyll and carotenoids also in the mature leaves.

CONCLUSION

It is apparent from these accounts that the ultimate effect of an air pollutant is on some important molecule(s) inside the cell. This effect changes the biochemistry of the cell to produce either "hidden" physiological effects or apparent injury. Before the air pollutant reaches inside the cell, it may also affect the stomatal movements and structure and function of cell walls and cell membranes. In many cases, effects on these structures bring about physiological, histological or morphological changes.

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EFFECT OF *IN VIVO* MUSCULAR STIMULATIONS

III. Some Aspects of Carbohydrate Metabolism of Cardiac Tissue

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ABSTRACT

The carbohydrate level of cardiac tissue was drastically decreased under the influence of *in vivo* muscular stimulations. The aminoacid content increased to a large extent. On successive muscular stimulations for 10 days the cardiac tissue appeared to have undergone a shift towards aminoacid oxidations, decreasing the utilization of carbohydrates.

INTRODUCTION

ELECTRICAL stimulation has been widely employed for inducing the muscular exercise[1-2]. Increased levels of oxidative enzymes of all tissues of the body[3] and of myocardium[4], have been reported following prolonged *in vivo* muscular stimulations and conflict stress respectively. Physical exercise also leads to increase in the oxygen demand of myocardium[5], and increases heart rate, regional blood flow and arterial blood pressure[6-7]. The values of these parameters were shown to decrease within 5–7 days of exercise. Since heart is known to involve in rapid activity during exercise, an attempt has been made to understand the possible changes in carbohydrate metabolism of cardiac tissue during muscular stimulations of short duration and prolonged periods.

MATERIAL AND METHODS

Frogs belonging to the species *Rana hexadactyla* (Lesson) were employed in the present investigation. Right gastrocnemius muscles of intact frogs were stimulated with electronic stimulator (INCO/CSIO Research Stimulator—Ambala) as described earlier[3] with a series of impulses (biphasic) of 5 V at a frequency of 120 pulses/min for 30 min per day for one day in one batch of experimental animals and for 10 successive days in another batch. The duration of each impulse was 100 ms, while the delay was 400 ms.

The cardiac tissue was isolated from freshly pithed control as well as experimental frogs and placed in amphibian Ringer to recover from shock effects. The heart was squeezed and washed thoroughly with amphibian Ringer to remove the traces of blood and taken for biochemical assays.

The activity levels of SDH, MDH and LDH were estimated by the method of Nachlas *et al.*[8] and GDH activity by the method of Lee and Lardy,[9] modified as follows. The reaction mixture in a final volume of 2 ml contains 40 μm of substrate (sodium succinate for SDH, sodium malate for MDH, sodium lactate for LDH and sodium glutamate for GDH) 0·1 μm of NAD (for MDH, LDH and GDH only), 100 μm of pH 7·4 phosphate buffer and 2 μm of INT (2-4 Iodophenyl) 3 (4 nitrophenyl) 5 phenyl tetrazoliumchloride). The reaction was initiated by the addition of 0·5 ml of the tissue extract. The incubation was carried out for 30 min at 37 C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight in cold with 5 ml of toluene. The intensity of colour was read at 495 mμ against toluene blank and the activity was expressed as μm of formazan/gm wt/hr. Total