PART III
Chapter I

GENERAL SUMMARY AND CONCLUSION
The study of fungal respiration especially those of pathogenic forms is obviously of great importance both from the viewpoints of fundamental and applied aspects. The characteristics of fungal respiration not only provide a valuable information with regard to the pattern of oxidative metabolism of the test organisms but also the experimental results, especially of the effects of various substances on the endogenous respiration of pathogenic fungi, may be profitably exploited in the selection of more effective chemotherapeutic agents. Besides this, the respiratory responses induced by chemotherapeutic agents very often reveal the valuable clues with regard to the mode of action of some of these fungitoxic substances particularly those which involve the oxidative metabolism of the sensitive organisms as the main target of their action. In view of this the present investigation was undertaken in which the respiratory characteristics of 4 plant pathogenic fungi have been studied by manometric techniques using Warburg's apparatus.

A detailed study of respiratory characteristics of *Gloeosporium papayae, Colletotrichum capsici, Rhizoctonia solani* and *Fusarium oxysporum* has been made which includes the effect of carbohydrates, amino acids, plant growth regulators, metabolic inhibitors, antibiotics, fungicides and culture filtrates of
various fungi on the rate of oxygen consumption by the mycelial suspension of test pathogens. Simultaneously for the purpose of correlation, the effects of above-mentioned substances on fungal growth of all the test organisms were also observed. Prior to these studies, to standardize the methodology the effects of age and starvation were also investigated so as to get better respiratory responses to the exogenous substrates.

Effect of age of the culture:

Five-day-old cultures of *G. papayae*, *R. solani*, and *F. oxysporum*; and four-day-old cultures of *C. capsici* were found to show maximum respiratory rates. However, after this period the respiratory activity gradually declined becoming approximately half of the maximum peak after ten days of incubation period. To avoid variations in the results due to cellular aging a period of maximum respiratory activity was chosen for all the determinations.

Effect of starvation period:

With regard to the effect of different starvation periods, it was found that the addition of glucose at the time of low endogenous rate (obtained after about 30 minutes of starvation) resulted in an immediate rise in the rate of oxygen consumption. It was evident that when *QO₂* values were reduced by starvation
then only the significant increase in the rate of oxygen consumption upon the addition of glucose was observed, therefore, prior to respirometric determinations the fungal mycelial mats of each test organism were kept in phosphate buffer for 30-40 minutes, so as to get better respiratory response to the exogenous substrates.

**Effect of carbohydrates:**

The rate of oxygen consumption in case of *G. papayae* was enhanced by seven carbohydrates with the maximum stimulation of 61% shown by xylose which was followed by trehalose, arabinose, mannose, galactose, fructose and glucose in their respiratory stimulation activity. The growth of *G. papayae* was supported by most of these compounds.

The endogenous mycelial respiration of *C. capsici* was considerably enhanced by lactose, ribose, xylose and glucose. Mycelial growth of the fungus was supported by lactose, xylose, ribose, glucose, raffinose and sorbose.

In case of *R. solani* galactose showed significant amount of activity by causing 174% stimulation in the rate of oxygen uptake. Rhamnose and fructose were also found to stimulate the rate to a considerable extent. Respiratory responses to others were, however, less significant. The fungal growth was supported well by arabinose (267%) and ribose (100%). Rest of the other
carbohydrates were less effective.

Respiratory stimulation in *F. oxysporum* was noticed by most of the carbohydrates with the maximum activity of 176%, and 161% shown by glucose and galactose respectively. The fungal growth was supported well by glucose, raffinose, galactose, xylose, lactose, arabinose and rhamnose.

In general, the respiratory and growth responses to carbohydrates are correlative to each other. A better activity shown by xylose than glucose observed in case of *G. papayae* indirectly indicated the presence of pentose-phosphate-pathway in the oxidative metabolism of this fungus. The unusually high stimulatory effect of arabinose on the rate of respiration in *R. solani* could be correlated with the recent work of Bateman *et al.* (1969) who have reported the involvement of an enzyme 'arabinase' during pathogenesis caused by *R. solani*. The abnormally high stimulation in the respiration and growth caused by glucose in case of *F. oxysporum* is rather remarkable because this carbohydrate was found to reduce the virulence of the pathogen (Baldin and Corden, 1969; Patil and Dimond, 1967), though, it may be explained on the basis of findings of Patil and Dimond (1968), who found that during pathogenesis, glucose treatment reduced disease symptoms while increases culturable mycelium of the fungus.
Effect of amino acids:

With regard to the effect of amino acids on oxygen uptake both stimulatory and inhibitory responses were noticed in case of *G. papayae*. Among stimulatory amino acids, threonine, L-proline and L-cystine were more active than others. Inhibitory effects of certain amino acids were, however, less significant excepting tyrosine which inhibited the rate up to 40% over control. The mycelial growth of *G. papayae* was considerably decreased in presence of tyrosine, DL-serine, and DL-valine.

In case of *C. capsici* the oxygen consumption was considerably enhanced by nine amino acids, with the maximum stimulation of 180% shown by DL-\(\alpha\)-alanine which was followed by DL-arginine, DL-methionine, DL-serine, DL-ornithine, glutamic acid, DL-aspartic acid, glycine and DL-valine in the stimulatory activity. Certain amino acids were found to produce inhibitory action. Among these tyrosine, threonine and L-proline were more effective than others. The mycelial growth was fairly supported by DL-arginine, DL-serine, and ornithine. The tyrosine was most effective for growth retarding activity which was followed by threonine, DL-\(\alpha\)-alanine and L-cysteic acid.

The endogenous mycelial respiration in case of *R. solani* was supported by most of these substances. Amongst them L-proline showed maximum stimulation causing 151% increase over control. Other significant stimulatory amino acids were DL-valine,
L-leucine, L-cysteic acid and L-cystine. $\beta$-alanine and tyrosine were found to inhibit oxygen uptake up to -77% and -32% respectively. The fungal growth was supported well only by DL-valine, glycine, DL-arginine and DL-lysine. The growth retarding activities shown by certain amino acids were, however, less significant.

Most of the amino acids in case of *F. oxysporum* were found to increase the respiration rate. Amongst the L-proline, L-cysteic acid, L-leucine, glycine, threonine, DL-serine and $\alpha$-amino-n-butyric acid were more effective than others. Certain amino acids have also retarded the rate, of which DL-arginine and tyrosine were more prominent. The fungal growth was supported by most of these substances, with the maximum stimulation of 164% shown by L-proline.

These results indicated that all the amino acids were not utilized as respiratory and growth substrates, however, the respiratory and growth responses to certain of these compounds are correlative to each other. In general the over-all effects of amino acids may be categorised in certain groups:

A. Strong stimulatory amino acids for both the processes; oxygen uptake and growth (DL-arginine, DL-ornithine, DL-serine in case of *C. capsici*, DL-valine in case of *R. solani*; glycine, L-proline and L-cysteic acid in case of *F. oxysporum*). These amino acids served as
best substrates for the organism concerned.

B. Strong inhibitory amino acids for both the processes were threonine in case of C. capsici, \( \beta \)-alanine in case of R. solani and tyrosine in all the test organisms. These amino acids appear to be fungicidal in nature. The fungicidal nature of certain amino acids has also been reported by Van Andel (1966).

C. The oxygen consumption was considerably enhanced but the growth was suppressed or not increased proportionately by some of these compounds, i.e., DL-\( \alpha \)-alanine, and DL-methionine in case of C. capsici, and L-cysteic acid, L-cystine, L-leucine and L-proline in case of R. solani. This may probably be due to their uncoupling action on fungal respiration. Earlier, the possibility of uncoupling action of some of these compounds has also been emphasized by Vyas, Saksena and Jain (1972).

**Effect of plant growth regulators:**

With regard to the effect of plant growth regulators on oxygen uptake and growth of test pathogens, it was noticed that in general these substances were found to cause the progressive stimulation in both the processes at lower concentrations while at higher concentrations they exerted adverse effects.
Effect of metabolic inhibitors:

Eight known metabolic inhibitors in different concentrations were tested against respiration and growth of all the test pathogens. The methylene blue produced adverse effects on both the processes which indicated the existence of a 'methylene blue' sensitive oxidative metabolism in G. papayae, C. capsici and F. oxysporum. Sodium azide inhibition as observed in case of G. papayae, C. capsici and F. oxysporum may probably be due to the fact that this inhibitor is known to form complexes with metals in cytochrome oxidase, due to which the transfer of electron to oxygen is prevented consequently, the rate of oxygen uptake may be decreased. However, in case of R. solani sodium azide has failed to produce adverse effects. This may be correlated to the findings of Van Etten et al. (1965) who have reported that R. solani has some tolerance to these inhibitors. Mercuric chloride, potassium ferricyanide and 8-hydroxy-quinoline have produced expected inhibitory effects on both the fungal processes. These inhibitors were found to exert significant inhibition at comparatively higher concentrations, as has also been reported by Vyas et al. (1972), Vyas (1971), Zentmeyer (1943). Sodium fluoride (which is known to inactivate the enolase enzymes of EM glycolytic pathway) was found to retard respiration and growth of C. capsici and F. oxysporum which indicated the presence of EM pathway in these organisms. In case of R. solani this inhibitor failed to produce significant
respiratory inhibition which may probably be due to the capacity of the fungus to resist this poison (Tolmsoff, 1962). Both the processes of *G. papayae* were slightly stimulated by sodium fluoride which do not indicate the presence of EM pathway as of prime importance in this fungus. These results can be correlated to that of Greene (1969) who reported that the prime route of respiration would most likely be the pentose phosphate pathway in *Gloeosporium musarum*. Sodium fluoroacetate has produced expected inhibitory action on both the processes of all the test pathogens, thereby indicating the presence of TCA cycle in the mechanism of respiration as it is known to exert toxicity by combining with oxaloacetic acid (Lièbecq and Peters, 1948, 1949; Buffa *et al.*, 1951) and to inactivate the aconitase enzyme activity (Lotspeich *et al.*, 1952). Sodium malonate inhibited the rate of respiration of *G. papayae*, *C. capsici* and *F. oxysporum*. As this inhibitor is known to act as competitive inhibitor of succinate dehydrogenase (Quastel and Wooldridge, 1928; Quastel and Wheatley, 1931) the presence of TCA cycle in the oxidative metabolism of these fungi was evident. However, in case of *R. solani* the rate was slightly stimulated which may probably be due to the fact that sodium malonate is also known to act as carbon source in certain cases (Clark and Wallace, 1958; Navak, 1959a, b). However, the presence of TCA cycle in *R. solani* as evidenced by the results with sodium fluoroacetate may be correlated with the findings of Van Etten
et al. (1965) who have demonstrated the presence of TCA cycle enzymes in the cell free extract of *R. solani*.

**Effect of antibiotics:**

With regard to the effects of antibiotics on respiration and growth of the test pathogens, out of the seven compounds, terramycin and streptomycin stimulated the oxygen uptake and retarded the fungal growth in case of *G. papayae*. Other antibiotics showed moderate or poor inhibitory effects on both the processes. In case of *C. capsici* amphotericin, streptomycin, gramicidin and terramycin have caused respiratory stimulation and growth inhibition. However, neomycin inhibited the oxygen uptake considerably. The oxygen uptake in case of *R. solani* was enhanced by streptomycin, terramycin, nystatin and amphotericin but simultaneously the growth was not supported by these substances excepting nystatin. In case of *F. oxysporum* only penicillin was found to show significant effect by causing 40% inhibition in oxygen uptake over control. Like respiration growth was also inhibited up to the same extent (-44%) by this antibiotic.

The over-all effects of antibiotics on respiration and growth did not show any correlation. In certain cases the respiration increased considerably but the growth was not supported or rather inhibited. It is quite probable that these antibiotics acted as uncouplers of respiration. Some of these
substances which were found to show strong inhibitory effects on mycelial respiration and growth may be important from the chemotherapeutic point of view as these can be used for more effective control (measures against pathogen concerned, i.e., streptomycin and terramycin in *G. papayae*, *C. capsici* and *R. solani*, and gramicidin and amphotericin in *C. capsici*).

**Effect of fungicides:**

Out of ten fungicides tested thiovit, cosan, thiram and captan were found to show significant antifungal action against *G. papayae*, in which both the processes were suppressed considerably. In case of *C. capsici* thiram, blitox, cosan, captan and thiovit showed encouraging results. The oxygen uptake by the mycelial suspension of *R. solani* was significantly decreased by captan, thiram, thiovit, blimix and cosan; however, the fungal growth was suppressed significantly only by thiram. Both the processes in *F. oxysporum* were suppressed by most of the fungicides with the maximum suppression of 60% shown by captan. It was evident that action of almost all the fungicides was similar on both the fungal processes excepting in case of *R. solani* where only thiram could produce significant amount of inhibitory effect on growth while on respiration five fungicides have been found to produce adverse effects. The poor inhibitory action of brassicol on respiration and growth of *R. solani* is of much interest as this fungicide has been reported to be most
effective against this pathogen (Ko and Farley, 1969). However, this poor inhibitory activity of brassicol might be due to acquired tolerance to the concentration used of the fungicide by *R. solani* (Thomas, 1962; Elsaid and Sinclair, 1964; Geogopoulos, 1963).

In other cases the strong respiratory and growth inhibition shown by certain of these substances such as thiovit, thiram and captan in *G. papayae*; captan and cosan in *C. capsici*; captan, cosan, thiram and sultaf in *F. oxysporum* appears to be due to their fungitoxic action. Though in case of *G. papayae* blitox, blitane, blimix and brassicol have produced significant growth inhibition without similar respiratory inhibition, which indicated that these fungicides might be acting on the phases of metabolism other than oxidative metabolism.

**Effect of culture filtrates of various fungi:**

With regard to the effect of culture filtrate of various fungi on respiration and their antagonistic action on test pathogens, the culture filtrates of *A. candidus* and *P. nigricans* were found to show strong stimulation in the rate of fungal respiration in all the four test organisms. For antagonism the test organisms were studied against the antagonists in plate experiments. *A. candidus* and *P. nigricans* showed strong antagonistic activity. This indicated that the respiratory stimulation might be due to the presence of some uncoupling agent being produced in the culture.
filtrates of these two fungi. The culture filtrates of certain fungi have produced good antagonistic activity with similar inhibitory action on oxygen uptake of the test pathogens indicating the presence of some metabolic inhibitor in the culture filtrates of the fungi concerned.

Trichoderma viride showed good antagonistic activity against R. solani while the respiration was not significantly inhibited by the culture filtrate of T. viride, which indicated that either antagonism of T. viride does not involve the antibiosis or if antibiotics are involved then the mode of action may be on the phases of metabolism other than respiration. Recently Dennis and Webster (1971) have reported that T. viride gives out volatile inhibitors which act antagonistic to many fungi, these substances obviously fail to remain in the culture filtrates till the tests for their effects on respiration are conducted.

The culture filtrates of three strains of A. terreus produced non-correlative effects on both the processes of test organisms, which indicated that these strains are biochemically quite different from each other.