DISCUSSION

The mechanism by which phytochrome displays its action on plant development is yet unknown. Since development in biochemical terms is an orderly sequence of change in enzyme complement and modulation of activities of certain key enzymes, the role of phytochrome in enzyme regulation has been much investigated. The mechanism by which phytochrome controls activity of enzymes is currently under intense debate (Mohr, 1972; Smith, 1975; Schopfer, 1977). In present investigation, we have endeavoured to decipher mechanism of action of phytochrome in controlling peroxidase activity in plants.

Dependence of Photoreponse on Organ and its Age

Phytochrome enhances peroxidase activity in apical leaves of 6-day-old etiolated maize seedlings (Sharma, 1974). In present investigation, this response was characterized in detail. Phytochrome regulation of peroxidase activity is found to be age dependent (Table 1). There is no enhancement in enzyme activity in 4-day-old seedlings. The photoreponse becomes conspicuous in 5-day-old seedlings and increased in magnitude with age. The enhancement in enzyme activity is brought about by a brief irradiation of red light, while a far-red light irradiation of similar duration is ineffective. The reversal of red light mediated enhancement by subsequent far-red light fulfills the
operational criterion for involvement of phytochrome in this response (Mohr, 1972).

The effect of red/far-red light on roots demonstrated a greater degree of variability and modulation of enzyme activity is not significant (Table 2). It shows lack of effective phytochrome involvement in this organ, and points that the photoresponse is organ specific. The age dependence and specificity of photoresponse supports the hypothesis advanced by Mohr (1972) that expression of Pfr-triggered photoresponse depends on 'specific state of differentiation' of particular tissue or cell. In Sinapis alba, phytochrome mediated increase in peroxidase activity is specifically confined to cotyledons and tap root, while in hypocotyl there is an inhibition (Schopfer and Plachy, 1973).

Pfr-mediates through Activators or Inactivation—a Comment

Phenolics are one of the factors which may regulate peroxidase activity in vivo. Russell and Galston (1969) reported that red light mediated modulation of level of phenolics could account for change in the level of IAA-oxidase activity in vivo. They proposed that phytochrome regulates growth by controlling synthesis of cofactors of oxidizing enzyme which catalyzes destruction of auxins. Phenolics also create nuisance during extraction of enzymes, as on oxidation they inactivate many enzymes irreversibly
(Loomis, 1974). This problem is overcome in our experiments by use of insoluble PVP in extraction buffer which adsorbs these phenolics (Loomis, 1974). The presence of such phenolic inhibitors and/or activators were also checked by additive experiments (data not shown). On mixing extracts from dark-grown and light grown plants, enzyme activity was always additive, thereby ruling out the possibility that enhancement in enzyme activity results due to change in level of activators or inactivators.

**Localization of Peroxidase**

Phytochrome mediated enhancement in peroxidase activity may result from either due to transfer of enzyme in different subcellular components or increase may be confined to some subcellular components. Peroxidase in apical leaves is mainly soluble enzyme, the other subcellular fractions had very little activity as compared to the final supernatant, obtained at 150,000 x g (Table 3). This observation is similar to Plesnicar et al. (1967) who reported that about 90% of peroxidase activity is soluble and 6% is associated with microsomes in mungbean seeds. In horse radish roots too, 80% of peroxidase activity is found to be soluble/phase, while 20% is associated with cell wall (Liu and Lamport, 1974). Since the recovery pattern of enzyme activity in dark and far-red light grown seedlings are similar (Table 3), it rules out the possibility
of intracellular transfer of enzyme under influence of phytochrome. In radish, it has been reported, that β-fructosidase transfers from cytoplasm to cell wall in roots and hypocotyl under the influence of phytochrome, and cycloheximide inhibits the Pfr-mediated increase in enzyme activity, but does not affect the transfer (Zouaghi and Rollin, 1976). FR-light mediated increase in peroxidase activity is mainly confined to supernatant (Table 4), this also justify our use of supernatant to study the change in enzyme activity under influence of phytochrome.

Control of Peroxidase Activity by Phytochrome - A Case of Photomodulation -

The enhancement in enzyme activity in seedlings irradiated with continuous far-red light (Fig. 3), demonstrates the operation of 'High Irradiance Reaction' presumably to be operating through phytochrome (Hartmann, 1966; Mohr, 1972; Schopfer and Mohr, 1972; Schafer, 1976; Rabino et al., 1977). Under continuous far-red light the peroxidase activity increases after a lag period of 2 h and reaches a steady state level at 20 h (Fig. 3). The presence of lag phase before the increase in enzyme activity points that the effect of phytochrome is not instantaneous, but requires build up of a potential to initiate the photoresponse. The existence of lag also rules out the possibility of direct photoactivation of enzyme as reported for nitrate reductase in mustard (Johnson, 1976). On transferring 6.5-day-old
seedlings to far-red light, peroxidase activity increase after a lag of 2 h. This observation is contrary to the results of Penel and Greppin (1973), who noted an immediate modulation in peroxidase activity after red/far-red irradiation in spinach and also in membrane vesicles isolated from Cucurbita pepo (Penel et al., 1976). Most of phytochrome mediated enhancement in enzyme activity is preceded by a lag phase e.g., 2 h for inorganic pyrophosphatase in maize (Butler and Benett, 1969) and 3 h for ascorbic oxidase (Drumm et al., 1972) etc. Mohr (1972) assumes that the lag phase can be explained by the time necessary to make genes accessible to far-red irradiation, therefore, no lag phase can be detected in case of secondary irradiation. Huault (1974) has shown activation of inactive phenylalanine ammonia-lyase in cotyledons of Raphanus sativus, and suggests that in this case lag phase is related to the critical level of \( P_{tot} \), which is about \( 27 \pm 3\% \) of maximum content of \( P_{tot} \). The presence of lag phase is encountered only when \( P_{tot} \) content is below the critical level.

The termination of far-red light after 12 h of irradiation brings a drop in rate of increase in peroxidase activity, albeit it is maintained at a higher rate than the dark control. Since the termination of far-red irradiation also leads to decline in \( P_{fr} \) level due to \( P_{fr} \)-decay
(Butler et al., 1963), one can correlate enhancement in peroxidase activity and presence of Pfr in the system. In other words, enhancement in peroxidase activity requires continuous presence of Pfr in system. It is evident from kinetics of enhancement that Pfr-regulates peroxidase activity in maize leaves in a manner characteristic of photomodulation, while it is a case of photodetermination in Sinapis alba (Schopfer and Planchy, 1973). A similar case of photomodulation has been reported for Pfr-mediated ascorbic acid accumulation in mustard seedlings (Bienger and Schopfer, 1970).

The continuous far-red light may not establish a stationary level of Pfr as predicted by Hartmann (1966) and Mohr (1972), because in maize the Pfr-decay is very rapid and get saturated at low energies (Schafer et al., 1975; Butler et al., 1963). Pfr-decay also depends on wavelength and at 750 nm it is slower than at 720 nm, however, level of Pfr is quite low at 750 nm (Schafer et al., 1975). Nevertheless, this low Pfr level controlled peroxidase activity to a significant extent. Since we have employed a filter emitting maximally at 750 nm, the Pfr-decay would have proceeded at a slow rate enabling us to detect HIR in controlling peroxidase activity.

The peroxidase activity remains stable in leaves as evident by kinetics of its activity (Fig. 3), once it is enhanced to a high level it does not decline back to the
level of dark control. Such stability of enzyme has earlier been observed for ascorbate oxidase (Drumm et al., 1972) and amylase (Drumm et al., 1971) while in case of PAL its level drops when Pfr is removed from the system indicating a rapid turnover (Dittes et al., 1971). The termination of far-red light could not completely stop increase in peroxidase activity, but the rate of enhancement is slowed down (Fig. 3). It appears that either peroxidase mRNA is stable to maintain peroxidase synthesis or rate of peroxidase turnover is slow.

Does Pfr controls through Hormones, Acetylcholine and c-AMP?

Hormones and phytochrome influence a variety of similar developmental processes in plants, still the participation of hormones in phytochrome mediated responses is controversial (Mohr, 1972; Smith, 1975; Wareing and Thompson, 1976). Hormonal regulation of enzyme activity has been studied in detail, for example, in case of GA induced amylase formation in barley aleurone layers (Mann, 1975). The involvement of hormones in phytochrome regulated enzyme activity, however, has not been investigated in detail. In the present study we have made an attempt to study the role of hormones, ACH and c-AMP in phytochrome mediated regulation of peroxidase activity in maize. Any particular hormone, in order to qualify as a mediator in phytochrome action, should satisfy the following criteria:
1. The *in vivo* titer of hormone should be either controlled by phytochrome and/or phytochrome should modify its distribution, transport or change the affinity of target of action.

2. Exogenously supplied hormone should mimick the action of phytochrome and kinetics of response induced by exogenous hormone should be similar to the kinetics of photoresponse.

3. In case phytochrome does not alter *in vivo* titer of hormone but change the affinity of target of action than in presence of exogenous hormone phytochrome action should either be enhanced or repressed.

4. The inhibitors which are known to affect the hormonal level or counter their action should nullify the phytochrome response.

In the present investigation, we have not studied the first criterion. Phytochrome control of hormonal level, transport and distribution are, however, well known (Black and Vlitos, 1972; Kandeler, 1974; Wareing and Thompson, 1976). In cereals, phytochrome lead to an increase in extractable gibberellin activity (Loveys and Wareing, 1971; Beevers, 1970; Reid *et al*., 1968), and its transport out of etioplast (Evans and Smith, 1976; Cooke *et al*., 1975). The control of level of other hormones has not been investigated in cereals, but such changes have been reported for other systems.
Regarding phytochrome mediated change in the affinity of target of action, it can be speculated on the basis of its association to particulate fraction in maize after red light irradiation (Quail et al., 1973).

When the other three criteria were studied, it was found that none of the hormones tested could absolutely be considered as a mediator in phytochrome controlled peroxidase activity in apical leaves of maize. The exogenous supply of GA, kinetin and ACH enhance the peroxidase activity in dark whereas c-AMP and IAA are ineffective (Table 5-7). The magnitude of enhancement is, however, always less than that obtained by 5 minutes of red light or continuous far-red light alone (Table 8). Moreover, the kinetics of enhancement obtained by these hormones are different and usually had a longer lag phase of 4 h, except GA, where it is of 2 h (Fig. 4-7) and the rate of enhancement is much slower than continuous far-red light, which also has a shorter lag time of 2 h (cf Fig. 3). The results with ACH are different from those reported in spinach, where the enhancement in peroxidase activity after ACH or red and far-red light treatment is apparent after only a few minutes (Penel and Greppin, 1973). Gibberellin induces peroxidase in wheat half seeds (Pollard, 1969) and α-amylase in maize (Katsumi, 1970). Kinetin also enhances peroxidase activity in lens roots (Gasper and Xhaufflare,
1967), while protects in barley leaves (Sharma and Biswal, 1976).

In presence of 5 minutes of red light or continuous far-red light the effect of hormones, ACH and c-AMP was different than in dark (Table 8, Fig. 4-7). Except DB$_2$ c-AMP, in 5 minutes red light, all others have a slight enhancing effect on peroxidase activity. Under continuous far-red irradiation, the enhancement is inhibited by all these regulators except with GA, which has only a marginal effect. In most cases, the repression in rate of enhancement became apparent only in latter phase of far-red irradiation. In DB$_2$c-AMP, IAA and kinetin it is apparent after 8 h, whereas with ACH it starts after 4 h.

Hormone mediated repression of photoresponses has been reported earlier also in a few cases e.g. in pea, where both auxins and GA negated phytochrome mediated responses (Russell and Galston, 1969; Purves and Hillman, 1958). Similarly, phytochrome mediated enhancement of phenylalanine ammonia lyase, anthocyanin and carotenoid synthesis is shown to be blocked by exogenous supply of BRB-8, a synthetic growth regulator (Dierickx and Vending, 1973). In mustard seedlings, GA repressed, when supplied exogenously, the far-red controlled amylase activity (Drumm et al., 1971). With ACH too, a complex interaction with light treatment has been reported in moss callus (Hartmann, 1974). On the basis of
kinetic studies, Giudici de Nicola et al. (1975) also ruled out possibility of kinetin and c-AMP acting as intermediates in FR light-mediated amaranthin synthesis. These compounds enhance the far-red mediated amaranthin synthesis in *Amaranthus caudatus*, while repress it in *A. tricolor*. Red light mediated lycopene synthesis in tomato is reversed by GA and kinetin but ABA potentiated this effect (Khudairi and Arboleda, 1971). In the present studies also, since hormone, irrespective of their stimulatory effect on peroxidase activity in darkness, are repressing the enzyme activity under continuous far-red light, it seems that in latter case some secondarily processes are triggered by hormone application which compete with the far-red induced photoresponse, thereby limiting the rate of enhancement.

The application of chemicals, which oppose or interact with the action of hormones, also did not give any results in favour of participation of ACH, c-AMP or hormones in phytochrome control of peroxidase activity (Table 9). Eserine, an inhibitor of ACH esterase (Fluck and Jaffe, 1975) does not affect red light mediated enhancement. We have not tested the effect of AMO-1618, another inhibitor of ACH esterase, which is shown to stimulate the red light mediated uptake of $^{14}$C-acetate in mung bean root tip (Jaffe and Thoma, 1973). However, another inhibitor atropine, which blocks ACH receptors (Goodman and Gilman, 1967), also failed to affect the red light mediated enhancement of peroxidase.
activity, though in *Avena* coleoptiles it negated the action of ACH (Evans, 1972). Under continuous far-red light, both eserine and atropine repress the enhancement in enzyme activity. Theophylline, an inhibitor of phosphodiesterase (Robinson *et al.*, 1971), is ineffective in dark but inhibited far-red mediated enhancement.

Similarly, the inhibitors of hormones also had no definite effect. Red light enhancement of peroxidase activity is not found to be sensitive to ABA, at a concentration where it blocks the action of most hormones (Milborrow, 1974). CCC, an antagonist of GA biosynthesis (Denis *et al.*, 1965) has little effect under red light, which may be due to its inhibitory action on protein synthesis (Kinsman *et al.*, 1975). Under continuous far-red light the repression by ABA and CCC is never more than 20 to 30%. In mustard seedlings too, Bajracharya *et al.* (1975) could not find any specific role of ABA in phytochrome mediated photomorphogenesis. Inhibitors employed in present investigation do affect hormonal level in cereal leaves, e.g., the pretreatment with CCC strongly inhibited light mediated increase in gibberellins in cereal leaves (Reid *et al.*, 1968), similar results were also obtained with ABA pretreatment (Beevers *et al.*, 1970).

Light has been shown to promote the formation of catalase isozymes in *Sinapis* (Drumm and Schopfer, 1974),
and hormones can alter the activity of peroxidases by a qualitative and quantitative change in isozyme pattern (Gasper et al., 1973). In our experiments, no qualitative difference is discernible with respects to different treatments in anionic or cationic isozymes of peroxidase on polyacrylamide gels (Fig. 8).

The involvement of hormones in phytochrome mediated photoresponse can be unequivocally considered only when a particular hormone satisfies all the criteria mentioned before. In the present investigation, although some of the compounds enhanced the peroxidase activity in dark, they can not be considered as a mediator in phytochrome response because, 1) the enhancement in dark is always less than that obtained by 5 minutes of red light or under continuous far-red light alone; 2) the kinetics of enhancement are different with longer lag phase; 3) the inhibitors tested could not bring down the far-red mediated enhancement more than 20-30%. It is very likely, therefore, that the enhancement induced by hormones, and their interactions with phytochrome mediated photoresponse, results from their independent action on a similar process. For instance Gibberellic acid (Wasilewiska and Kleczkowski, 1976) and c-AMP (Tarantowicz and Kleczkowski, 1975) enhance RNA synthesis in maize seedlings. On the other hand, ABA inhibits nucleic acid metabolism and RNA polymerase activity in maize (Bex, 1972a,b; Tarantowicz and Kleczkowski, 1975), ABA also
affects membrane properties as it inhibits ion accumulation in maize (Shaner et al., 1975). Any generalization on participation of hormones, ACH or c-AMP on phytochrome mediated photoresponse should be, therefore, carefully interpreted and based on rigorous analysis and not merely by showing the mimicking effect e.g., as has been done for c-AMP (Rast et al., 1973; Weintraub and Lawson, 1972) and ACH (Penel and Greppin, 1973).

**Role of Photosynthesis in HIR**

The nature of photoreceptor(s) controlling HIR response has been a matter of much discussion. Some people support the idea that phytochrome is the sole receptor involved in HIR responses (Borthwick et al., 1969; Hartmann, 1966; Mohr, 1972), whereas other argue for existence of a second photochemical system besides phytochrome (Grill and Vince, 1970; Schneider and Stimson, 1971, 1972; Downs et al., 1965). There is some evidence that this second photochemical system involved in HIR could be photosynthesis (Creasy, 1968; Downs, 1964; Downs et al., 1965; Schneider and Stimson, 1971, 1972).

**I. Effect of Photosynthetic inhibitors** - One of the principle argument in favour of participation of photosynthesis in HIR is effectiveness of photosynthetic inhibitors to block FR-mediated HIR (Schneider and Stimson, 1971, 1972). The inhibitors of cyclic PP like DNP and antimycin-A and
noncyclic PP like DCMU and $\text{NH}_4^+$ brought a reduction in enhancement in peroxidase activity mediated by far-red light (Table 10, 11). Schneider and Stimson (1971, 1972) proposed that in FR-mediated HIR besides phytochrome, cyclic PP also participates, on the basis of inhibition of FR-mediated anthocyanin synthesis by inhibitors of cyclic PP. Their proposal is, however, based on data obtained by Arnon et al. (1967) on effect of these inhibitors on PP in isolated chloroplasts. Therefore, the use of these inhibitors may not represent a true picture, as in vivo effect of these inhibitors may be considerably different from in vitro studies. In fact, these inhibitors stimulated respiration to some extent in treated seedlings in our studies (Table 12), though significant difference could not be seen. This may perhaps be due to use of excised leaves, as excision itself stimulates respiration, thereby making effect of inhibitors. It has been shown that these inhibitors are not specific in vivo and they do effect other cellular processes. In Chlorella for example, DNP at a concentrations which inhibits endogenous oxidative phosphorylation and increase the endogenous respiration maximally do not inhibit photosynthesis (Kandler, 1958). Furthermore, both DNP and DCMU inhibit RNA and protein synthesis (Gruenhagen and Moreland, 1971; Moreland et al., 1969). Antimycin-A is a potent inhibitor of oxidative phosphorylation (Pomeroy, 1975; Storey, 1972) and $\text{NH}_4^+$ may affect respiration by blocking citric acid cycle.
(Wakiuchi et al., 1971). The specificity of inhibitors is also questioned by Mancinelli et al. (1974), while studying far-red mediated increase in anthocyanin in cabbage and mustard seedlings.

II. Development of photosynthetic pigments in continuous far-red light - As the participation of photosynthesis in FR-mediated HIR can not be conclusively demonstrated by use of inhibitors, we have therefore alternatively studied the development of photosynthesis under continuous far-red light and compared it with the normal development under white light. Mohr and his associates (Mohr, 1972; Masoner et al., 1972) ruled out the possible contribution of photosynthesis in FR-mediated HIR in mustard seedlings, as there is no significant chlorophyll development under FR light. However, in maize as we can measure there is a substantial increase in chlorophyll level under FR light although it is much less in comparison to chlorophyll level under white light (Fig. 9, 10). The slow chlorophyll accumulation under far-red light is obvious as wavelengths only in the blue and red region are most effective, while far-red is least effective in mediating chlorophyll development (Koski et al., 1951). The characteristic peak at 663 nm of extract of pigments in 80% acetone confirmed their identity as chlorophylls (Fig. 11). There is no lag in chlorophyll development, both chlorophyll a and b developed simultaneously.
Chl. b development was slower in FR light than in white light as evident by high Chl a/b ratio under FR light. Shylk et al. (1970) have also obtained similar results in maize seedlings that Chl. b appeared within a few minutes of initial transformation of protochlorophyll. On the other hand, in bean (De Greff et al., 1971; Weistrop and Stern, 1977), no Chl b development took place until 48 h of far-red irradiation. Besides chlorophyll, FR light also influenced development of photosynthetic accessory pigment carotenoids (Fig. 12). Red/far-red reversible control for carotenoid synthesis has been reported for maize seedlings (Cohen and Goodwin, 1962). Our observations confirm this by further demonstrating the operation of HIR in controlling carotenoid level.

Although there was substantial development of chlorophyll under continuous far-red light, there seemed to be no direct relation of this response with the increase in peroxidase activity by far-red light. In our experiments, the exogenous application of chloramphenicol in maize, though inhibits chlorophyll biosynthesis (Table 13) does not affect peroxidase activity under far-red light (Table 14). Similarly, another greening inhibitor, actinomycin D (Bogorad and Jacobson, 1965) also has no significant effect on peroxidase activity (Table 14). A similar conclusion is reached by Mancinelli et al. (1974, 1975) by using chloramphenicol and streptomycin which inhibit chlorophyll biosynthesis
but enhance FR light induced anthocyanin formation. Schneider and Stimson (1972), however, have shown that inhibition of chlorophyll biosynthesis by levulinic acid also inhibited FR-mediated HIR in inducing anthocyanin synthesis.

Since there is a significant development of photosynthetic pigments under FR light, it seems quite possible that functional development of photosynthesis may also take place under FR light. Wellburn and Wellburn (1973) showed that FR light triggers development and differentiation in isolated plastids in vitro. The absorption spectra of isolated plastids from leaves greened under far-red light shows a peak in red region at 673 nm, while isolated plastids from leaves greened under white light shows a peak at 678 nm and characteristic soret band in blue region (Fig. 13). The position of peak at 673 nm indicates the organisation of plastid is not complete in far-red light. This is evident by the fact that the peak position is at 678 nm in plastids isolated from white light-grown seedlings. During development to mature organelle this peak in red region always shifts towards 678 nm (De Greff et al., 1971), while treatments of mature chloroplasts to disorganization agents shifts peak toward the blue region (Biswal and Mohanty, 1976). In bean leaves greened under far-red light shows that although prolameller body disappears and thylakoids
are formed in plastids, there is no fusion of thylakoids to form grana unless plants are irradiated with white light (De Greff et al., 1971). It appears that though far-red light triggers pigment development, the plastids are not fully differentiated to be optimally active in photosynthesis. This is also evident by the fact that photochemical electron transport chain is not complete in far-red irradiated seedlings, as there is no light mediated $O_2$ evolution in vivo till 24 h of far-red irradiation, whereas this capacity develops at 8 h in light grown seedlings (Fig. 14). In bean leaves greened under far-red light, onset of photosynthetic $O_2$ evolution is detected after 24 h of irradiation (De Greff et al., 1971).

III. Effect of far-red light on development of photochemical activities - The onset and development of various photochemical activities under continuous far-red light was also studied by using isolated plastids. In all cases the development in photochemical activity is preceded by a lag phase. PSI develops after 2 h of irradiation under white light, while under far-red light it develops after 4 h (Fig. 15). Both under FR light and white light, PSII activities could be detected after 4 h (Fig. 15). The cooperation between two photosystems also develops along with the development of PSII (Fig. 17). The basis for expressing results of development in photochemical activities merit some discussion. The results in present
investigation are expressed both on the basis of chlorophyll and fresh weight of leaves. It is observed that whereas there is an enormous change in chlorophyll content with duration of irradiation, fresh weight does not change to the same magnitude. Furthermore, due to the difference in chlorophyll content in far-red light and white light grown seedlings, the activities can be better compared on fresh weight basis than on chlorophyll basis. When results are expressed on chlorophyll basis, the photochemical activities show a peak at 8 h, which decline at 16 h (Figs. 15-17) in white light-grown plants. Such a curve is not too evident when data are expressed on fresh weight basis. Under far-red light, however, such a characteristic peak at 8 h of irradiation is never noticed, however, activity of PSI and cooperation between PSII-PSI shows decrease with increase in amount of chlorophyll molecules. This occurrence of peak at 8 h in white light grown plants and declined of PSI and PSII-PSI activities in far-red grown plants may arise from different rate of synthesis of reaction center chlorophyll molecules and bulk chlorophyll molecules. It seems that reaction center molecules are synthesized predominantly in initial phase than the bulk chlorophyll molecules, thereby, resulting in high photochemical activity, when expressed on the basis of chlorophyll. However, during latter phase synthesis of bulk chlorophyll is faster than reaction center species, which lead to an apparent decline in photochemical activity. On
the basis of fresh weight, increase in activity is stationary between 8-16 hours of white light irradiation. Similar curves in photochemical activity development on chlorophyll basis was also observed in barley, while it was absent when results were expressed on basis of fresh weight (Egneus et al., 1972). It is difficult to decide at present, whether this rise in photosynthetic activity reflects a higher rate of synthesis of reaction center molecule as compared to bulk chlorophyll or change in other properties, (e.g., change in enzyme properties) associated with photosynthetic unit. The evidence supports the former view, since it has been observed by Faludi-Daniel et al. (1970) between 3-12 of greening of maize plastid, the synthesis of reaction center molecule P700 lagged behind synthesis of bulk chlorophyll. The cooperation in PSII-PSI remained at a constant level under far-red light on fresh weight basis, while PSI and PSII activity increased during same period (Fig. 15-17). It seems that some key component of electron transport chain is limiting in far-red grown plants, so cooperation remained at a steady state level. It is also supported by the fact that CO2-dependent -O2 evolution also could not be detected in vivo in far-red grown plants (Fig. 14), pointing to incomplete nature of electron transport chain.

In white light, both cyclic and noncyclic PP developed after 8 h of irradiation, while under far-red light cyclic
PP could be detected at 8 h, but noncyclic could be detected only at 24 h (Fig.18,19). Our results are not in agreement with earlier reports where cyclic PP could be detected within 1.5 h of white light irradiation (Forger and Bogorad, 1971; Arntzen, 1970). Oelze-Karow and Butler (1971) detected DCMU insensitive ATP changes in bean leaves after 12 h of far-red irradiation presumably representing cyclic PP in vivo, while DCMU sensitivity appeared at 24 h. However, such light induced change in ATP level in vivo may not necessarily be due to cyclic PP as light can also independently modulate ATP level as observed in bean (White and Pike, 1974; De Greff et al., 1976). The hypothesis elaborated by Schneider and Stimson (1971, 1972) is based on the fact that although spectrum of photosynthesis is localised in blue and red region, under FR light (> 700 nm) the cyclic PP mediated by PSI can operate. In Chlorella, it was shown that cyclic PP may provide energy for the induction of isocitrate lyase (Syrett, 1964). However, such participation of cyclic PP in enhancing peroxidase activity is not obvious, as under FR light cyclic PP develops much latter than the enhancement in peroxidase activity.

The occurrence of cyclic PP, its capacity and physiological significance is also uncertain and controversial (Heber, 1969, 1973). In particular, the published rates of endogenous cyclic PP of isolated intact chloroplasts are extremely low, e.g., about 0.2-5 μ moles/mg Chl·h, whereas rate of CO₂ fixation may reach 200 μ moles/mg Chl·h (Kaiser and
Urbach, 1974). High rates of cyclic PP have only been observed in vitro in the presence of suitable cofactors such as phenazine methosulfate (Tanner et al., 1969), in vivo cyclic PP furnishing ATP occurred under nitrogen, and Heber (1969) reported that in presence of oxygen it is suppressed and replaced by pseudocyclic electron transport. Heber (1973) further presented evidence that light dependent-increase of both chloroplasts NADPH and ATP is suppressed by DCMU at concentrations which are ineffective in suppressing PSI-dependent cyclic PP of broken chloroplasts. Simnois and Urbach (1973) considered that cyclic PP may be a relic of evolutionary past and exists today preferentially in lower plants. Mohanty et al. (1971) showed that in Porphyridium cyclic PP is absent in mature cells, but may be present during early developmental stages. Since enzyme enhancement occurs predominatly in cytoplasm (Table 4), the participation of cyclic PP in such response would also require transport of generated ATP to cytoplasm, but high energy compounds like ATP and NADP can not cross the plastid membrane barrier in vivo (Heber, 1974). Only compounds which can be exchanged are 3-phosphoglyceric acid and dihydroxy acetone phosphate, whose generation depends on active CO₂ fixation by plastids, which is absent in seedlings grown under far-red light, as there was no light mediated CO₂-dependent-O₂-evolution (Fig. 14).
Our results on development of various photochemical activities are in agreement with earlier reports. The development of PSI activity preceded PSII activity, however both cyclic and noncyclic PP develop at the same time in white light. Butler (1965) using light induced fluorescence changes demonstrated that PSI was active before PSII, in greening bean leaves. In greening bean leaves, Hill reaction could be detected in 5-10 h but NADP reduction appeared only after 16 h (Gyldenholm and Whatley, 1968). In this case cyclic PP appeared at 8 h, while noncyclic PP appeared at 10 h.

In another study on bean leaves both cyclic and noncyclic PP could be detected at 3-4 h, but efficient coupling to electron transport chain was achieved only after 15-18 h (Howes and Stern, 1973). In barley BSI activity appeared within five minutes of illumination, while PSII activity appeared after 3 h of illumination (Egnerus et al., 1972).

In isolated plastids from far-red grown bean plants, cyclic PP was detected by 8 h, while noncyclic electron transport and PP could only be measured after 12 and 16 h of far-red irradiation respectively (Weistrop and Stern, 1977). It seems that there is quite a variation in different species in onset and subsequent development of photochemical activity, furthermore, age and cultivar difference may also affect sequence of development.

It's evident from the above discussion that photosynthesis plays no role in FR-mediated HIR regulating peroxidase activity in maize. The onset and development of all photochemical activities under far-red light lag behind
the FR-mediated enhancement in peroxidase activity. The participation of PSI-mediated cyclic photophosphorylation in HIR can not be favoured, as it develops much later than increase in peroxidase activity. It seems that photosynthesis has no role in FR-mediated HIR at least in etiolated maize seedlings and the effect of phytochrome on peroxidase activity and photosynthetic activity development are quite independent of each other.

Pfr-regulation of Peroxidase Activity at Transcription or Translation level

I. Effect of inhibitors - One obvious prediction of differential gene activation hypothesis is de novo synthesis of enzyme molecules in positive photoresponse under influence of Pfr. Evidence for this has come mainly from use of the inhibitors of transcription (Carr and Reid, 1966; Mohr and Bienger, 1976; Dittes et al., 1971; Drumm et al., 1971) and translation (Drumm et al., 1971, 1972). These inhibitors either reduce or prevent the phytochrome effect. In present investigations chloramphenicol an inhibitor of organelle protein synthesis, do not affect Pfr-mediated enhancement in peroxidase activity (Table 14) thereby ruling out the possibility of participation of organelles ribosomes in this response. The ineffectiveness of actinomycin-D to repress the enhancement of peroxidase activity suggests that there is no major participation of transcription (Table 14). The phytochrome-mediated enhancement in peroxidase activity in
the present system probably results from its synthesis on cytoplasmic ribosomes, as puromycin completely prevented the enhancement (Table 15). In sweet potato, actinomycin-D inhibited RNA synthesis by 60-70%, but did not block increase in peroxidase activity (Matsushita and Uritani, 1975). These authors interpreted that either actinomycin-D fails to repress mRNA synthesis for peroxidase, or peroxidase is synthesized on preformed mRNA which has relatively longer half-life. Similar suggestions were also made for isolated wheat embryo by Taneja and Sacher (1976), in this system, while actinomycin-D (100 μg/ml) failed to repress peroxidase activity, cycloheximide (10 μg/ml) repressed it by 82%, at this concentration actinomycin-D inhibited RNA synthesis by 70% while cycloheximide inhibited protein synthesis almost completely. In our investigations also while actinomycin-D blocks RNA synthesis significantly (Table 21), it had very less effect on peroxidase activity (Table 14), indicating for a possible existence of a stable pre-mRNA of peroxidase.

The enhancement in peroxidase activity in presence of cycloheximide (Table 16) suggests the existence of proteinaceous inhibitor of peroxidase with rapid turnover rate, however, alternative interpretations are also possible. Cycloheximide probably inhibits the synthesis of this inhibitor thus resulting in an enhancement in peroxidase
activity due to reduction in its rate of inactivation. The differential response noted at different concentrations of cycloheximide may be due to its differential effect on synthesis of peroxidase and inhibitor protein. At low concentration (5 \( \mu g/ml \)), cycloheximide may stop inactivation but may not hinder the synthesis of peroxidase thereby resulting in enhanced activity. This may be the reason for the differential increase observed in peroxidase activity in plants kept in darkness and those treated with far-red light (Table 15). At higher concentration (20 \( \mu g/ml \)) of cycloheximide, enzyme synthesis may also be inhibited thereby leading to an overall reduction in magnitude of enhancement. An almost identical response was noted in sunflower discs by Creasy et al. (1974), where on comparing cycloheximide mediated inhibition at different concentrations they showed that inactivation was much more sensitive to cycloheximide than the phenylalanine ammonia-lyase formation. From the kinetics of enzyme activity in presence of cycloheximide and continuous far-red light it seems that they do not have a common mode of operation (Fig. 20). Cycloheximide probably increased peroxidase activity by inhibiting enzyme inactivation, while in far-red light fresh enzyme synthesis takes place. The stationary level of peroxidase after enhancement by cycloheximide further supports the contention that peroxidase is a stable enzyme and does not decay during the course of investigation (Fig. 20).
Suggestions for the presence of inactivators, regulating enzyme activity, have been made for other systems too. These inactivators are classified as binding inhibitors, specific proteinases and modifying enzymes. Binding inhibitors are known for potato invertase (Matsushita and Uritani, 1976; Pressy, 1967) and phenylalanine ammonia-lyase (French and Smith, 1975). Wallace (1973) showed the existence of specific proteinases inactivating nitrate reductase in maize. The results of Blondel et al. (1973) suggest the existence of some modifying enzymes for phenylalanine ammonia-lyase.

Oaks and Jonson (1973) found that cycloheximide causes an accumulation of glutamine in maize roots. Glutamine in certain cases has been reported to affect activity of certain enzymes, e.g., asparagine synthetase (Rognes, 1970) and peroxidase (Dezsi et al., 1970), and cycloheximide may act as an antagonist of glutamine (Ross, 1974; Jones, 1977; Oaks and Johnson, 1973). These observations prompted us to investigate a possible relationship between cycloheximide-mediated enhancement and glutamine. Exogenous glutamine enhances the enzyme activity in etiolated seedlings maximally at 5 mM (Table 17), however, Dezsi et al. (1969) got enhancement in peroxidase activity at 150 mM. The enhancement in peroxidase activity is greater in presence of glutamine in far-red irradiated seedlings, but this effect is neither synergistic nor additive (Table 18). Cycloheximide does not act as an antagonist to
glutamine since at equimolar concentrations cycloheximide mediated enhancement is not prevented by glutamine (Table 19). In maize roots cycloheximide mediated inhibition in asparagine formation was relieved by exogenous glutamine (Jones, 1977). The possibility of cycloheximide-mediated change in glutamine level affecting peroxidase activity requires further investigation.

The use of inhibitors although suggests possible involvement of nucleic acid and protein synthesis, but by no means provides a proof for such a mechanism as the in vivo specificity of these inhibitors is questionable. In fact cycloheximide shows too many side effects in vivo e.g., inhibition of ion uptake, respiration (Ellis and MacDonald, 1970) and many other metabolic processes (McMahon, 1975). Similarly, chloramphenicol also inhibits ion uptake and oxidative phosphorylation (Ellis, 1969) and energy transfer in chloroplasts (Wara-Aswpati and Bradbeer, 1974).

II. Effect on DNA and RNA synthesis - The effectiveness of inhibitors of protein synthesis in repressing Pfr-mediated enhancement in peroxidase activity suggests a possible role of protein and nucleic acid synthesis in phytochrome action. In seedlings irradiated with continuous far-red light, DNA content in leaves is not changed (Fig. 21), whereas RNA level
increased after a lag phase of 2 h, reached its maximum at 8 h, then gradually declined, at a slower rate than the control (Fig. 22). This decline may result from the change in the rate of RNA synthesis and/or degradation. Gyurjan (1974) reported that white light had no effect on DNA level in maize leaves while it increased RNA synthesis, particularly of plastid rRNA. Weidner (1967) also could not detect change in DNA content in mustard cotyledons under far-red light while rRNA level increased in both cytoplasm and plastids after a lag period of 6 h and 12 h respectively (Weidner and Mohr, 1967; Thien and Schopfer, 1976). In barley leaves, synthesis of rRNA of cytoplasm precedes that of plastid rRNA, in light (Poulson and Beevers, 1970). In the present investigation we have not characterized the nature of the RNA components influenced by far-red irradiation, nevertheless existing studies suggest that it is mainly localised in rRNA.

In contrast to continuous far-red light a brief red irradiation does not bring about significant change in the RNA level (Table 22). Such changes have been reported for mustard cotyledon (Weidner and Mohr, 1967) and pea buds (Jaffe, 1969). In pea buds after brief red irradiation, rRNA level increased after 2 h to a maximum at 6 h (Koller and Smith, 1972). The efforts pertaining to demonstrate change in mRNA has so far met with failures (Dittes and Mohr, 1970).
however, a change in tRNA was reported (Okoloko et al., 1970). In maize, Harel and Bogorad (1973) after 2.5 h of light treatment detected a new RNA species presumably mRNA. A clear demonstration of phytochrome control of mRNA is still awaited, however, light mediated change in level of PAL mRNA has been reported (Ragy et al., 1977; Schroder, 1977). Phytochrome mediated increase in RNA level may result from a change in RNA polymerase activity. Light mediated changes in RNA polymerase activity have been reported for barley (Poulson and Beevers, 1970) and pea (Ellis and Hartley, 1971; Bottomley, 1970) and plastid and nuclear polymerase of maize (Bogorad, 1967; Stout et al., 1967).

III. Effect of Pfr on polyribosome formation - The synthesis of proteins takes place on ribosomes, which aggregates on mRNA to form polyribosome complex. The amount of polyribosomes, in a cell or tissue, is, therefore an indirect indication of rate of operation of protein synthesizing system. In present study, far-red light induces polyribosome formation after 2 h lag and it increase by 10% over the control by 8 h (Fig. 23, 24). Furthermore, polyribosomes isolated from FR grown plants incorporates $^{14}\text{C-L}$ lysine into proteins more actively than those from the dark control (Table 23). Our results on polyribosome level though different from earlier ones (Travis et al., 1970),
can be easily explained since we isolated total polyribosomes, comprising of membrane bound, free and organelle polyribosomes by the inclusion of Triton X-100, a membrane disrupting agent in homogenizing medium, while Travis et al. (1970) isolated only free cytoplasmic polyribosomes.

Nevertheless, results on the effect of continuous far-red light on polyribosome level and activity are quite comparable to the ones reported for maize seedlings. William and Novelli (1964) reported that white light enhanced protein synthesis in isolated polyribosomes in maize after a lag of 1.5 h to a maximum at 3 h and remained unchanged till 11 h. In a further study, they reported that white light-mediated increase in the polyosome level was accompanied with increased \textit{in vitro} leucine incorporation (Williams and Novelli, 1968). A lag phase of 2 h preceded the white light effect on \textit{in vitro} incorporation of $^{14}$C-leucine into protein, and of different light treatments, red light was most effective. Travis et al. (1970) also reported light-mediated increase in polyribosome level in maize, which was correlated with increase in activity of nitrate reductase (Travis and Key, 1971). Red light also activated monoribosomes in maize by increasing the level of peptidyl tRNA associated with ribosomes (Travis et al., 1974). It is very evident from these studies that light plays an important role in regulating protein synthesis by controlling polyribosome level in
maize and that the action of far-red light is very similar to that of white light. Since in our studies too, the lag period for enzyme induction was roughly equivalent to the lag found for polyribosomes formation, it seems that phytochrome mediated increase in peroxidase activity may result from the increase in polyribosome level. It is also apparent from our earlier experiments, that peroxidase mRNA may be a stable one. The presence of stable mRNA in seed development is also well documented (Weeks and Marcus, 1971) and similar suggestion was advanced for stored peroxidase mRNA in wheat embryo (Taneja and Sacher, 1976). The enhancement in peroxidase activity may result from increased level of polyribosome leading to increased rate of translation of mRNA of peroxidase. Travis and Key (1971) also speculated same type of translational control for light-mediated induction of nitrate reductase in maize leaves.

Recently, Smith (1976) has advanced a model suggesting translational control by phytochrome of protein synthesis on the basis of studies conducted on bean and other plants. In bean, inhibition of mRNA synthesis by cordycepin did not affect the light-mediated development of polyribosomes, and the lag phase (1 h) of polyribosome development was shorter than that of RNA synthesis (2 h). In a recent publication (Giles et al., 1977), they have further shown
that light mediated enhancement in protein synthesis may be due to mobilization of stored mRNA into polyribosomes in bean leaves. Although the classical operon concept of Jacob and Monod (1961) argued that all regulation of gene expression should be at the transcriptional level for bacterial system. Sufficient experimental evidences have now been accumulated that in eucaryotes, amount of enzyme in tissue may be a direct result of post transcriptional control. The results obtained in present investigations also suggest some post transcriptional control for Pfr-regulation of peroxidase level in maize leaves.