IV DISCUSSION

Under etiolated conditions the moisture content of Cucumis seedlings gradually increased from 2 to 14 days of growth along with rapid elongation of hypocotyl (2 to 6 days) up to a certain stage, followed by a slower rate (Fig 2c, Page 50). When growth rate per seedling was taken into account, or less a gradual increase in fresh weight with an increase in moisture content and dry matter was observed up to 8th day followed by a decrease (Fig 2a, Page 50) till 14 days.

Total phenol content per seedling increased simultaneously with an increase in fresh weight and decreased with a decrease in fresh weight during growth (Fig 2a, b; Page 50). However, when expressed on unit fresh weight the phenol level decreased with increase in fresh weight of seedling till 4th day. Between 4 and 6 days of growth the content increased with increase in fresh weight (Table III, Page 37). Subsequently there was a decrease on fresh weight basis. Thus apart from those fluctuations the phenol level per unit fresh weight continuously decreased during growth. The initial fluctuations on fresh weight could be attributed to differences in moisture content of seedling.

The fluctuations in all the individual phenolic acids parallel with those of total phenol. Chlorogenic acid was in highest amounts among all the phenolic acids (Fig 3b, Page 44). Caffeic, p-hydroxybenzoic and e- coumaric acids were the next
highest (Fig 3c, e and f; Pages 45, 46) p Coumaric and ferulic acids were the lowest in content in etiolated seedlings of Cucumis (Fig 3a and d; Pages 44 45)

The high gibberellin content in the early stages was associated with a low phenol level and high phenol content was related to low gibberellin content in the later stages. The lowering of growth rate during later stages can be attributed to a low gibberellin content and due to relative levels of phenolics and endogenous gibberellins. A drop in the amounts of endogenous gibberellins was observed with rapid elongation (Fig 4a Page 55)

As regards the individual gibberellin like substances it was noticed that the amounts of all of them decreased gradually with advancement in age (Fig 5 1 7) (Pages 58, 59)

Auxinoxidase activity was at a lower level during the initial stages (Table VII, Page 79) of seedling growth where high amounts of the chlorogenic acid were present with a high level of gibberellins

The fact that of all of the individual phenolic acids chlorogenic acid was in highest concentration is quite interesting (Fig 3b; Page 44) High levels of chlorogenic acid was known to act as the inhibitor for auxin oxidase (Robins and Klein, 1957) This would agree with the present findings since low auxin oxidase activity was associated with high chlorogenic
acid level That the levels of individual phenolic acids play an important role in controlling growth rate by regulating the levels of auxin oxidase is confirmed by the present observations.

Thus as far as the etiolated seedling growth was concerned an inverse relationship of phenol level to auxin oxidase activity was observed between 2 to 8 days (Tables III and VII; Pages 39 and 79 respectively).

Murakami (1961) showed a gradual decrease in the amount of gibberellin like substances in Pharbitis seedlings with increasing age under etiolation. Wheeler (1960) also suggested that the gibberellin content of cotyledons of bean plant showed a gradual decrease in gibberellin content after reaching its maximum on 4th day. He demonstrated same phenomena with whole seedlings.

Polyphenoloxidase level fluctuated parallel with phenol level (Table VII, Page 79).

With age under natural conditions the rate of growth by fresh weight per seedling steadily increased more or less linearly up to 14 days. Then it rose to its maximum abruptly till 18 days after germination (Fig 2d, Page 52). The dry weight of the seedling also showed the same trend with growth up to 18 days. Rapid growth of seedling by elongation was noticed during 4 to 6 days, thereafter a linear increase in length of hypocotyl was observed till 18 days of growth (Fig 2a, Page 52).
Th sudden increase in growth by fresh and dry weight of dilute phenol solution during 14 and 18 days can be attributed to the formation and expansion of leaves.

An increase in phenol content was significant with the pan of dilute phenol solution used. The high strength of phenol solution was found between 12 and 18 days (Fig. 2f; Page 52) during which period the growth rate was very rapid. The total phenol content increased simultaneously with increasing fresh and dry weights during growth both in seedling and unit fresh weight. The growth rate of seedlings exhibited a direct relationship with each other. The content of phenol in solution during 16 to 18 days of the highest weight of seedling was detected in a 10% solution in the unit plant mass (Table III; Page 37).

It is noted that during seedling growth under natural conditions the increase in phenol content is ascribed to a more or less co-occurring increase in auxin oxidase activity. The seedlings grown under natural conditions showed an inverse relationship between phenol content and auxin oxidase activity on unit fresh weight (Table III and VIII; Page 37-80). These findings are in support of the hypothesis of Garay et al. (1959) who...
also showed similar relationship

The increase in phenol content with age under natural photoperiod was apparently an expression of light induced formation of these compounds in leaves. The effect of light on the formation of phenolic compounds was demonstrated by Hillis and Swain (1957) who showed that a higher phenol content was present in the leaves of *Prunus domestica* on the sunny side than in the leaves on the shady side. This aspect was further stressed by Zucker and Ahrens (1958) because of the necessity of light to form chlorogenic acid in potato tubers. More precise evidence of role of light was presented in the experiments conducted by Bayer (1961) and Masuda (1962). They have estimated the inhibitor content of seedlings in the etiolated conditions and after exposure to light and they could obtain a marked increase in inhibitor content in the seedlings which were given light treatment. Phillips and Wearing (1959) suggested that short photoperiods favour the synthesis of inhibitory substances than under longer photoperiods. The present investigation also supports the statement set forth by earlier workers regarding the importance of light for phenolic biosynthesis.

Light induces morphogenetic effects such as suppression of elongation of seedling and promotion of leaf expansion. These photomorphogenetic effects could be explained on the basis of relative amounts of individual phenolic acids as evidenced by ti
present work p Coumaric acid was found to be the chief constituent among all the phenolic acids in the light grown seedlings and the same known to be an activator for auxin oxidase (Gortner and Kent, 1958). Since the level of phenolic activator of auxin oxidase was high (Fig 3a, Page 44) the rate of oxidation of auxin would also be rapid. Thus lesser amounts of auxin will be spared and accordingly elongation will be suppressed. On the other hand, in darkness chlorogenic acid an inhibitor of auxin oxidase was in high level which spares more auxin and in turn leads to greater elongation.

With growth the level of endogenous gibberellin increased which attained its maximum on 18th day per seedling. The increase in phenol level could possibly be due to the endogenous gibberellin level. A direct relationship between the levels of endogenous gibberellin and phenolics was established (Table III and Fig 4b; Pages 37, 55 respectively). Unlike in the etiolated seedlings, the seedlings grown in natural photoperiod exhibited a direct relationship between phenol level, growth rate and gibberellin level.

The individual gibberellins also showed the same trend as the total gibberellins (Fig 6, Pages 63, 64).

The auxin oxidase activity increased parallel with growth reaching its maximum on 18th day. Auxin oxidase levels correspond with the levels of p-coumaric acid which activates the oxidase.
thus bringing a over 11 upper side in the long time as dugling in light a composed of dark green dillig. The increased auxin xidased activity during growth in the seedlings grown in natural day light than in the seedlings grown in etiolated conditions added to the growth put forth by Stutz (1957) He showed in his auxin xidase activity in the seedlings grown under natural day light than in those grown in etiolated conditions. This would seem to indicate that the presence of finding in auxin xidase levels much higher (20 to 40 fold) under natural conditions than in the dark. Although chlorogenic acid was present in their xidase activity unlike in etiolated seedling where it was less in the light grown dillig because of higher levels of p-cumaric acid. The auxin oxidase activity was thus enhanced in light.

That auxin oxidase activity may regulate growth by ntrillings in fumitory 3 acetic acid has been shown by several authors. The relationship of light period in which seedlings were grown and to auxin xidase activity was shown by Gay et al (1959) and Stutz (1957). Sagl and Garvey (1961a) have also suggested a correlation between auxin oxidase activity and phenolic content in Lupinus leaves.

Peroxidase was believed to be an essential part of the auxin oxidase system as suggested by Galston and Dalberg (1954). In the present work the activity of auxin oxidase was estimated as
peroxidase Occurrence of peroxidase as the actual component of indoleacetic acid oxidase was also demonstrated in number of plant tissues Jensen (1955) measured the activity of peroxidase in root section of *Vicia faba* and found it strongly inducible by a treatment with IAA.

An exogenous supply of gibberellic acid (at 100 PPM level) under natural conditions did not cause any significant increase in weight At the same time neither the level of phenol nor auxin oxidase were affected (Tables III and VIII; Pages 37 and 80 respectively) But the endogenous gibberellin level was increased by the gibberellin treatment (Fig 4c, Page 55)

The lack of effect of gibberellic acid on the seedling is probably due to high endogenous gibberellin level during early stages Galston and Warburg (1959) and Watanabe and Stutz (1960) in pea stems and *Lupinus albus* respectively showed that gibberellic acid enhances the level of auxin oxidase inhibitors which are generally accepted as the phenolic compounds The present investigation in contrast to these findings did not show an increase in phenol level

Gibberellin treatment enhanced the elongation process (Fig 2c, Page 52) If the mechanism of growth promotion by exogenous gibberellic acid is through auxin spraying action the levels of auxin oxidase and its inhibitors would have been altered However, such an effect was not noticed here Hence the exogenous growth does not seem to be operated through levels
of IAA. On the other hand, the endogenous growth appears to be controlled through levels of auxin oxidase. Sirois and Parup's (1965) showed that exogenous GA promotes the level of endogenous growth promoting substances and also reduces an acidic inhibitor. The present work agrees with their findings in as much as the increase in endogenous gibberellin was found here also.

The effect of gibberellic acid on auxin oxidase activity was studied by several workers. Lowered auxin oxidase activity was observed by Pilet and Wurgler (1958); McCune and Galston (1959) also showed a lowering of peroxidase activity in the tissues treated with gibberellin while others have found no change in the enzyme activity (Kato and Katsumi, 1958; Kogl and Sterna, 1960). Brain and Hemming (1958) were also unable to observe any effect on auxin oxidase activity by GA in young Lupinus plants. On the other hand, Hayashi et al. (1956) found an increase in the activity of peroxidase by gibberellin treatment. In the present investigation no such effect of gibberellin was found on the activity of auxin oxidase during growth. With an increase in fresh weight gradual increase in enzyme activity was noticed.

The intensity of light and length of photoperiod given to seedlings are known to show marked differences in the formation of endogenous phenolics, gibberellins and on auxin oxidation.
through auxin oxidase. Light and low temperatures exert some morphogenetic effects through control of endogenous growth regulators. 7 days after germination the GA treated seedlings were transferred from natural photoperiod conditions (day light alternating with night) to a static condition of continuous illumination. With age the phenol content decreased from what it would be in natural photoperiod (Table III, Page 37). More or less a constant activity of polyphenoloxidase was maintained (Table VIII, Page 80). Although phenol level was much less than under natural conditions, a markedly high auxin oxidase level was present here (Table VIII, Page 80). Thus continuous low intensity illumination increases auxin oxidase activity.

Generally short photoperiods favour synthesis of phenolic substances. Contrary to this Garay et al. (1959) showed an increase in the concentration of phenolics in the plants exposed to natural day light alternating with 16 hour continuous illumination under cool white fluorescent light. But since in the present investigation the seedlings were subjected only to a 24 hour illumination of dull light (1600 lux) there was no accumulation of phenols.

also showed the reduction in the content of inhibitory substances when the plants were subjected to low temperatures. The phenol content decreased on being transferred to 26°C.

The GA treated seedlings on being transferred to continuous darkness where more elongation of seedlings was observed than under natural photoperiod with chlorotic symptoms. The phenol content decreased very much from that in natural conditions (Table III, Page 37). The gibberellin content also decreased (Fig. 91, 6, Page 75, 76). Low gibberellin content can be related to low phenol level. Auxin oxidase was highest (Table VIII, Page 80).

As regards the mobilization and translocation of phenolic substances within the seedling the study of individual parts of Cucumis seedlings has revealed certain interesting observations. With unit mass the different parts showed a gradual decrease in phenol content during growth (Table IV, Page 39). Roots and cotyledons possessed high phenol content than hypocotyls during growth. The occurrence of a lower phenol content in the hypocotyl would suggest a translocation of substances present in cotyledons to the growing regions. As in the case of whole seedlings grown under etiolation, the phenol level decreased up to 8 days in different organs due to increase in growth rate by moisture content. There was a gradual accumulation in the roots after 8th day at the expense of the cotyledonary phenol content.
since there was a corresponding decrease in the cotyledons
Thus an increase in the content could be observed in roots during
growth. High phenol level in roots and cotyledons during early
periods of growth (2 days after germination) was associated with
a high polyphenol oxidase activity. There was then a decrease
in activity accompanied with a lower phenol level. The activity
of auxin oxidase showed an inverse relationship to phenol level.

The phenol levels in different organs of onion plant at
various ages also suggest a translocation from the site of rapid
synthesis. When leaves were formed on the bulbs phenolic syn-
thesis would increase and a rapid translocation takes place to
other parts thus increasing the amounts of phenolics in root and
bulb portions. An increase in the synthesizing organ, the leaf
also occurred during growth (Table V, Page 41). Until leaves
were not formed a gradual increase in the phenol content was
observed in roots which suggests a probable translocation of
phenolics from bulbs to the roots. During growth the bulb also
showed an increase in phenol content even when leaves were
absent which can be attributed to a synthesis of phenolics
possible from carbohydrate precursors. After leaf formation
a rapid synthesis could occur thus translocation of substances
from site of synthesis results in accumulating compounds in other
organs of the plant such as roots and bulbs. The increase in
phenol content in the roots, bulbs and leaves with increasing
age agrees with the observations of Varga and Koves (1959).
Significant qualitative changes in the phenolic acid composition during growth in individual organs has occurred (Table II, Page 32). In the initial stages p-coumaric acid was absent in the roots. Absence of p-coumaric and caffeic acids in 9 day old root suggests that it might have been converted into ferulic and sinapic acids. The same was true with p-coumaric acid in bulb also. Lack of p-coumaric acid which can be accounted for its rapid conversion into other cinnamic acid derivatives. Even when the fresh synthesis of substances does not take place simple mobilization of compounds can occur.

A decrease in phenol level from apical leaves of Coleus towards leaves at the base (Table VI, Page 43) suggests that younger leaves contain larger amounts of phenols when compared to old. This would suggest that during growth the level of phenolics had decreased. As the young leaves are synthesizing more compounds at the apical region the high levels of phenolics would help in lignification. This might suggest a translocation of substances from the rapidly synthesizing young leaves to differentiating zones where these phenolic compounds play an important role.

The experiments conducted with $^{14}CO_2$ also revealed the same pattern of rate of biosynthesis and age of organ. The young leaves of Coleus were capable of fixing more carbon 14 into phenolic fraction than the older leaves. With increasing age
of leaf the total chlorophyll as well as phenol content decreased with increase in fresh weight of leaf. Decrease in phenol level was also observed by Griffiths (1958) with respect to age of leaves in Theobroma.

The results obtained in this investigation support the view, that phenolic substances and other related compounds are formed in a photosynthesis because the importance of light in the formation of phenolic compounds was already mentioned. Racusen and Aronoff (1954) clearly shown that detached soyabean leaves did not synthesize phenylalanine and tyrosine in darkness from $^{14}$CO$_2$. Phenylalanine and tyrosine are the good precursors of phenolic compounds. Stone (1953), Brown et al (1953) have studied the formation of lignin in wheat plants during the stage of rapid lignification with $^{14}$CO$_2$. They found the formation of lignin precursors via shikimic acid pathway. This also agreed with the findings of Kreitsberg and his associates with Populus trichocarpa (Brown, 1964). The formation of lignin precursors such as phenylpropanoid units was studied by McCalla and Neish (1959) who found that shikimic acid is a good precursor of lignin. During photosynthesis the intermediates of carbon reduction cycle such as tetrose phosphate may be drained away for the synthesis of shikimic acid (Calvin and Bassaham, 1962).

The formation of caffeic acid and chlorogenic acids as the major phenolic compounds from $^{14}$CO$_2$ shows the involvement of
shikimic acid pathway in phenolic biosynthesis (Neish, 1960)
The synthesis of phenolic in Coleus leaves was also to some extent dependent upon the amount of chlorophyll. In the present investigation experiments were conducted with the leaves while they were attached to the plant. The chlorophyll content per unit weight of material in mature leaves decreased when compared to young leaves (Table VI, Page 43).

The formation of protocatechuic acid was another evidence for the involvement of shikimic acid pool. Protocatechuic acid has been suggested by Gross (1955) to be produced by the dehydration of dehydroshikimic acid. Since the intermediates of the shikimic acid pool, caffeic, chlorogenic, p-coumaric and protocatechuic acids were all noticed to be formed during assimilation of carbon dioxide by Coleus leaves, shikimic acid pathway of aromatic biosynthesis is taken to be confirmed. The occurrence of only traces of p-coumaric acid on autoradiograms (Fig 10, Page 86) suggest that it is an intermediate in the synthesis of caffeic acid. The enzyme system capable of converting p-coumaric acid may be highly active so that caffeic acid is formed rapidly.

The degree of cell differentiation in terms of lignification could be correlated with phenol content in seedlings of Cucumis melo. At about 14 days high rate of lignification occurred when also a high phenolic acid level was there. Thereafter the
content was decreased simultaneously with the lignification. This would suggest a direct relationship between phenol synthesis and the lignification.