CHAPTER IX

GENERAL DISCUSSION.

This investigation was carried out with a design to have fuller appraisal of the chemical factors responsible for leaf expansion. The seedlings were raised in the dark since under etiolated conditions response to chemical factors is generally better. The etiolated leaves being devoid of chloroplasts, cannot efficiently synthesize the food materials (sugar in particular) necessary for growth and development in consequence of which the seedlings maintain nourishment from the reserve food contained in the seeds. The seedlings were allowed to grow under such conditions for 8 days. After being robbed of their endogenous growth substances, the leaves develop hunger. It was expected that this sort of experimentation would help to give a unified picture of the broad spectrum of the externally added food factors and lead us one step ahead to a solution of the problem of the exact role of organic and inorganic substances in the physiology of leaf expansion.

Organic substances, particularly carbohydrates constitute the bulk of the food factors for building up cell-wall besides being an energy source. Mineral nutrients including both macro- and micro-elements also play
important role in the growth and development of leaves. The vitamins and amino-acids synthesized abundantly by normal leaves are translocated to other parts of the plant for their utilization. Feeding the etiolated leaves with exogenous carbohydrates, vitamins, amino-acids and mineral nutrients would therefore pose an interesting problem for investigation. Auxins and allied substances such as gibberellic acid, kinetin etc., all are potent growth stimulators especially in the aerial organs of plants. Anti-auxins and inhibitors are also known to influence the growth of intact or isolated plant parts in a variety of ways. Therefore, it would be a pertinent problem for investigation as to the nature of action of these organic and inorganic compounds on the expansion growth of leaf disks isolated from etiolated bean seedlings.

The interesting feature to be noted is that an isolated growing segment of a plant organ is a constantly changing system from the moment of excision to the cessation of growth particularly in its response to external agencies besides undergoing change in structure and metabolism. This point was thoroughly scrutinised by Audus (1952) in his time-factor studies on growth inhibition in excised organ sections. The situation becomes far more complicated in sequel to the addition of exogenous
growth substances in the medium. It is, therefore, con-
tended that magnitude and time relationship of the growth
rate curve of such a system are of fundamental importance
and the observation of a rapidly growing system at a regu-
lar interval of time would give a clue to this changing
system rather than observation only once after the lapse
of a definite time period. Keeping in view Audus's idea,
the present investigation was designed to make measure-
ments after regular interval of time and working out the
growth rate subsequently in case of experiments with car-
bohydrates.

Difficulty is often experienced in studying
the nutritional requirements of a culture medium. It is
difficult to compare this requirement to vivo expe-
riments since the compounds necessary for the growth and
development of particular intact organ may be made avail-
able through regular transport from the focal point of
its synthesis lying at a distance. Free cells or tissue
explants may lack some essential metabolite in a given
biosynthetic sequence. This difficulty may be overcome
by supplementing the culture media with various exogenous
compounds. However, attempt by several workers to estab-
lish a strictly defined medium for culturing cells or
tissue explants did not culminate in desirable success.
Our preliminary experiments (results not incorporated in
the thesis) revealed that leaf tissues failed to survive for longer span of time in sterile distilled water. The necessity for an energy source in the culture medium was felt very urgently. All throughout the investigation 1% sucrose solution was used as the basal medium except for experiments with carbohydrates. Sucrose, besides being an energy source, supported good growth continuing more or less at an equal pace during different time intervals. Bacterial and fungal growth usually observed with relatively higher concentrations of carbohydrates is reduced to the lowest degree at this concentration of sucrose. The other advantage of this concentration of sucrose is that it maintains a continuous growth of lower magnitude which can be modified by the addition of other compounds. Thus, the study of the effect of added compounds becomes easier in sucrose medium.

The five carbohydrates tried included two disaccharides, two monosaccharides and one pentose sugar. Sucrose promoted disk expansion significantly commencing from the first growth phase to the end of the experiment. The growth rate of sucrose treated disks reached the peak value during the first growth phase and then sloped down during the subsequent two phases. Maltose also registered highly significant stimulatory effect in this regard.
Glucose and fructose proved highly stimulatory within the same range of concentrations applied. All these four carbohydrates exhibited identical pattern of growth stimulation. A gradually ascending trend of stimulation from 0.5 to 5% (maltose 2.5%) was observed followed by growth decline at the next higher concentration. The optimal concentration stood at 5% except for maltose with its highest growth stimulation at 2.5% solution. The broad pattern of growth stimulation was identical depicting the highest rate during the first growth phase and then declining in the next two phases. Arabinose, the only pentose sugar tried proved to be ineffective.

This finding well illustrates the imperative need of carbohydrate in the culture media as an energy source for the sustained growth of the tissues. This lends support to the view expressed by Gautheret (1955) that the plant tissues must be cultured in media supplemented with sugar. The optimal range of concentrations fell within 0.5 to 5% thus corroborating Gautheret's (1942) and White's (1943) observations of the optimal range of sucrose and glucose of 2-5% in the culture of normal carrot tissues and isolated root-tips. Hilderbrandt and Riker (1953) observed well-marked optima in the range of 2-4%. In relative efficiency of all the carbohydrates tried, sucrose proved superior to all other, followed by glucose, maltose
and fructose. This is in conformity with the findings of Gautheret (1945), Hilderbrandt and Riker (1949, 1953), Street and Lowe (1950) and Henderson (1954) who reported the superiority of sucrose over all other carbohydrates in their experiments with various tissue explants. Arabinose proved ineffective for supporting growth of the tissues. The demand for utilizable carbohydrates appears to be same in all kinds of tissues as had been clearly evidenced from these findings with normal carrot tissues, crown gall tissues, root tissues and leaf-tissues.

The path of entry of the carbohydrates seems to be the cut edges of leaf-tissues as they are in direct contact with the solution. Such a view was put forward by Weatherley (1947) and Went and Carter (1948). From equimolar concentrations the rate of absorption of glucose and fructose is same (Weatherley, 1954). Now, from the spate of stimulation caused by these two monosaccharides it is clear that glucose is more efficient than fructose at least in the leaf tissues. Both are the constituents of cell-wall building materials being present within the cells in soluble state. Besides, they provide energy for the growing tissues. The fact that sucrose which combines glucose and fructose in its molecule is the best carbon source seems justified as in the process of degradation more energy is liberated. All these sugars
stimulated growth at all the concentrations tried. There was no appreciable growth inhibition even with very high concentration of the order of 10 per cent. Gautheret (1941) made similar observation in carrot tissue. The osmotic pressure of the solutions apparently control the rate of penetration of the solutes into plant cells. The growth rate during three intervals of time showed a general pattern of stimulation. The growth rate was higher during the first phase and sloped down in the next two phases. This growth trend can be attributed to the rapid absorption and utilization of carbohydrates during the first phase. The efficiency of absorption and utilization perhaps falls with the aging of the tissues coupled with depletion of endogenous growth factors. With the progress of time more cell-wall materials are deposited and this probably makes higher concentrations less demanding and less utilizable. These four carbohydrates proved to be the utilizable ones, probably because of their ability to undergo interconversion within the tissues which might satisfy the need of specific carbohydrate for tissue growth. Such interconversion of sugars was irrefutably demonstrated by Goris (1954) in carrot tissues. The negligible growth produced by the lowest concentration of arabinose might be at the expense of endogenous food materials which disappeared with the passage of time.
Mineral nutrients, potassium nitrate and cobalt nitrate used in the present investigation both stimulated expansion growth markedly. The overall growth promoting activity of potassium nitrate was found to be higher than that of cobalt nitrate. Both the salts contained same acid radical $\text{NO}_3^-$. The importance of nitrogen in plant metabolism needs no emphasis. Nitrate is beneficial for both the constituents - nitrogen and oxygen. Oxygen replaces deficiencies of it in the culture medium, whereas nitrogen imparts its manifold activities. Nitrogen probably enters the plant cells to take part in protein synthesis. Besides, nitrogen is incorporated in purine, pyrimidine, porphyrin and co-enzyme molecules. Purines and pyrimidines are indispensable constituents of nucleic acids, RNA and DNA and consequently both the acids control protein synthesis in the cells. Porphyrin group controls the synthesis of chlorophyll and cytochrome enzymes which are essential for plant metabolism, such as photosynthesis and respiration. Moreover, nitrogen is associated with vitamin synthesis. It is beyond doubt that the expansion growth of the leaf disks were controlled in part by nitrate radical of both the mineral salts.

On comparative assessment of relative efficiency of $K^+$ and $\text{Co}^{++}$ it appears that the latter is about fifty times more efficient than that of $K^+$ in bringing
about maximum leaf disk expansion. The conclusion was derived from the observation of optimal concentrations of KNO$_3$ (500 p.p.m.) and that of cobalt nitrate (10 p.p.m.). Potassium being a macro-element is probably needed in larger quantities than cobalt. Potassium affects various processes such as respiration, photosynthesis and chlorophyll development. Deficiencies of potassium provokes disturbances in normal functioning of these vital processes for growth. Potassium is closely associated with the activity of certain enzymes involved in synthesis of certain bonds (Webster, 1953, 1954, 1956) as well as in carbohydrate metabolism. This finding is in conformity with those of Bonner (1940) and Miller (1951) who observed the effectiveness of KNO$_3$ in leaf expansion. On the other-hand, the less demand for CO which is a micro-element seems quite probable. Concentration of 10 p.p.m. proved to be optimal under our experimental conditions. The effectiveness of cobalt in promotion of growth of pea root-sections (Galston and Siegel, 1954; Das, 1954), Avena coleoptile sections (Thimann, 1956; Busse, 1959) and etiolated pea stem sections (Somner, 1961) had also been reported. Miller (1951) applied four salts of Co$^{++}$ with different acid radicals to test the relative efficiency in leaf disk expansion. He observed that at 2 and 5 p.p.m., the extent of expansion was the same. He considered this
promotion of disk growth as being due to the basic radi-
cal Co\(^{++}\) and not the acid radicals. Since, in the pre-
sent investigation, the acid radical is the same, the
observed difference in activity is due to the basic radi-
cal factor. While potassium is known to have involved in
so many metabolic processes as mentioned above, cobalt is
known to participate in the synthesis of vitamin B\(_{12}\). How-
ever, it is unlikely that vitamin B\(_{12}\) stimulated growth,
since this vitamin is ineffective in promoting plant
growth (Thimann, 1956). Miller (1952) observed identical
action of cobalt and that of light in bringing about leaf
disk expansion. Light and cobalt appeared to affect the
same sequence of reactions that limit the leaf growth. In
other words, this micro-element stimulated growth by
participating in the same vital processes as that control-
led by light. It seems quite likely that cobalt promotes
some step in oxidative metabolism which normally makes a
source of energy (perhaps ATP) available for growth and
divert it from other metabolic processes (Thimann, 1956).
Further, the presence of sucrose in the medium enhances
the growth stimulating activity of cobalt (Miller, 1954; 
Thimann, 1956). The present finding is in full agreement
with those of Miller (1951, 1952), Dale (1966) and Loer-
cher and Liverman (1964) who reported stimulation of ex-
pansion growth of bean leaf disks by cobalt. The growth
rate at the optimal concentration KNO₃ increased with 
the progress of time, while with Co(NO₃)₂ it reached the 
peak value during the second growth phase and then decli­
ned in the next phase. This depressed growth stimulation 
and the inhibition at higher concentration of cobalt might 
be due to inactivation of a sulfahydryl group (Thimann, 
1956).

**Vitamins.** The three vitamins - thiamine hydrochloride, 
pyridoxine hydrochloride and nicotinamide were tried with­
in a concentration range of 0.01 to 100 p.p.m. Both thia­
mine and nicotinamide exhibited highly significant stimu­
ulatory effect, while pyridoxine failed to reach that level.
The former two did not inhibit growth even at the highest 
concentration of 100 p.p.m., but pyridoxine imparted in­
hibitory effect during first growth phase. Das (1965) and 
Das and Das (1966) observed no stimulation of pea root 
sections with dilute concentration of thiamine. This wide 
difference in results of thiamine may be due to the dif­
fERENCE in response of two widely different organs from 
which the explants were excised. The action of pyridoxine 
seems to be not different. Hilderbrandt et al. (1945) ob­
served slight growth stimulation by pyridoxine. The low 
degree of growth stimulation induced by pyridoxine might 
be due to its synthesis in large amounts by the cultured
tissues as envisaged by Czosnowski (1952) for which the need for exogenous one is much less. The present investigation does not rule out the essentiality of pyridoxine as a stimulatory agent. The leaf tissues may evoke great demand for thiamine as had been evidenced by its highly significant stimulatory effect. There occurred slight depression in stimulation at the highest concentration of thiamine in late hours. Probably with progress of time, the cells became over-saturated with vitamin in consequence of continuous penetration into the cells resulting in growth depression. The leaves are the seat of synthesis of vitamins. The leaf tissues for that reason probably could withstand the highest concentration of thiamine, and consequently marked growth stimulation ensued. This is the consensus of opinion of several workers (Peters and O'Brien, 1955; Bonner, 1942c; Bonner and Dorland, 1943a, b) who observed thiamine in large amount in youngest leaves. Thiamine-induced growth stimulation is probably due to its participation as a co-enzyme in the decarboxylation of L-keto-acids. Thiamine was also probably synthesized by cultured leaf disks, but their increasing demand could not be fully met for which they had to depend upon exogenous supply. Pyridoxine within the tissues undergoes conversion into pyridoxal and pyridoxamine, which are then phosphorylated to give pyridoxal phosphate.
and pyridoxamine phosphate. Pyridoxal phosphate probably participated as a coenzyme in amino-acid metabolism. Transamination and decarboxylation reactions are catalyzed by pyridoxal phosphate. Nicotinic acid is also reported to be synthesized by leaf tissues in light (Crane, 1954, a,b). The present investigation was carried out in dark and probably this made the tissues dependent on exogenous supply of the vitamin. Even if some amounts were synthesized they could not satisfy the need of the growing leaf tissues. The similar view was expressed by Åberg (1961) that addition of niacin stimulates growth due to suboptimal rate of its biosynthesis. Nicotinamide at all the concentrations tried did not inhibit growth. Galston's (1949) report on the bud growth of etiolated pea plant is in conformity with its behaviour to cultured leaf tissues. Nicotinic acid functions through its two forms of coenzymes, NADP and NAD in dehydrogenase system bringing about oxidation in cellular metabolism. Further, nicotinamide had caused stimulation through its interaction with endogenous IAA which resulted in additive effects. For further elucidation of this point IAA-nicotinamide interaction was studied. The interaction surface during the three growth phases revealed an additive effect of both the compounds acting jointly at lower concentrations. This is in line with the report of Galston (1949) for pea
epicotyl sections. This interaction might be due to identical action of both the compounds acting on the same site, since they are derived from a common precursor, tryptophan. It is probable that the addition of either compound makes available more tryptophan for conversion to the other, and thereby increases effectively the concentration of both the constituents even by the addition of one. This interconversion might have the following path-way as suggested by Galston (1949).

\[
\text{Tryptophan} \rightarrow \text{Tryptamine} \rightarrow \text{Nynurenine} \rightarrow \text{Hydroxyanthra-} \rightarrow \text{Nicotinic acid}
\]

After Galston (1949).

The additive effect of the lowest concentration NAm might be due to conversion to IAA bringing the latter to optimal level while the higher concentration could not induce such pronounced additive effect. But at the highest concentration too, inhibition was not well marked, probably some of NAm were functioning as coenzyme in cellular metabolism.
Amino-acid. DL-Alanine was found to stimulate growth significantly. Riker and Gutsche (1948) did not observe promotion of the growth of sunflower crown gall tissue by alanine. Inhibition was the general effect. This contrasting results of alanine in two different tissues may be due to their differential sensitivity. Alanine induced a well-marked growth promotion from the lower to higher concentrations with optimum peak at 50 p.p.m. Alanine probably combines with organic acid produced by the leaf tissues to form other readily utilizable organic nitrogenous substances leading to the formation of proteins which caused marked stimulation of growth.

Coumarin which is a potent growth inhibitor or germination inhibitor was found to stimulate expansion growth of leaf disks, recording 50 p.p.m. as optimal concentration. This finding is supported by the results of Miller and Meyer (1950-51) who observed similar growth promotion of Chenopodium album leaf disks by coumarin using a wide range of concentrations of 1-200 p.p.m. Thimmann and Bonner (1949) and Gantzer (1960) recorded synergistic effect between coumarin and IAA in the growth of oat coleoptile. Gantzer (1960) observed synergistic effect of coumarin in the presence of 10^{-5}M IAA but at higher concentrations this synergism was lost. Thimann and Bonner (1949) observed synergistic effect between lower concentrations
(10^{-6}, 10^{-5}, 10^{-4} M) of coumarin and IAA also on the growth of oat coleoptile sections. The higher concentration (100 p.p.m.) induced growth inhibition as revealed in the present investigation. This is in agreement with results of Thimann and Bonner (1949). Nitsch and Nitsch (1961) also observed synergism between phenolics and IAA. That coumarin stimulates growth in presence of adequate amount of endogenous auxin was also reported by Barlow et al. (1955) and Shibacka et al. (1957). Further, Andreas (1952) observed that another lactone, scopoletin could inhibit indoleactic oxidase. The growth stimulation by coumarin might be mediated either through auxin-sparing action as scopoletin does or synergism between coumarin and endogenous IAA which might had reached the level needed for such synergism.

Coumarin-induced growth stimulation was attributed to its carbohydrate breaking capacity resulting in the accumulation of cell-wall material precursors and acceleration of glycolytic phosphorylation (Knypl, 1964). Besides, Knypl (1964) observed that the longitudinal growth of sunflower hypocotyl sections was largely dependent on coumarin-induced synthesis of RNA, proteins and ATP. He further observed increased respiration of P. vulgaris leaf tissues as a result of coumarin application. Thus, the expansion growth of bean leaf disks in consequence of coumarin application might be due to a synergism with
endogenous IAA, or increased synthesis of RNA, ATP or proteins or increased respiration releasing large amount of energy which stimulates growth.

Skatole, an indole derivative lacks carboxyl group in the side-chain. According to Mc Rae and Bonner (1953, a,b) it is an anti-auxin identical with 2,4-dichloranisole. It bears the same relation to IAA, as 2,4-dichloranisole does to 2,4-D. This carboxyl free analogue of IAA was applied within a wide range of concentrations of 1-100 p.p.m. Skatole was found to be highly effective in causing growth stimulation, recording 10.40% increase at its optimal concentration of 1 p.p.m. during the last growth phase (48-72 hours). Thimann (1958) in his pea curvature test observed some promotion or synergism at the low concentration (1 and 3 x 10^-4 M) of skatole, while high concentration inhibited growth. But in pea straight growth experiment he observed general effect of growth inhibition. In the present finding, the higher concentrations were found to have imparted toxic effects causing inhibition. This inhibition went on piling up as time elapsed. Further, it recorded a lateral shifting of the optimal concentration from 2.5 p.p.m. to 1 p.p.m. after the first growth phase (0-24 hours). Skatole probably functions as does coumarin in blocking the activity of enzyme resulting in the rise of the level of endogenous auxin to optimum.
The activity of skatole supports the view expressed by Thimann (1958) that it functions as an inhibitor rather than an anti-auxin. Particularly in leaf tissues, it probably functions in an identical manner as does coumarin which is recognised as a potent growth inhibitor.

**Beta(2-Furyl)-Acrylic acid** is a substituted fatty acid. Acrylic acid is a volatile unsaturated fatty acid. It is present in plants either in free state or in combinations with formyl or acetal groups of sugars, and the polysaccharides and proteins and pectins are associated with them. (Scarisbrick, 1955). The furyl group is attached to the beta-position of acrylic acid which makes it more active. It was found highly stimulatory within a range of concentrations (1-5 p.p.m.) during all the three growth phases. But the inhibition induced by higher concentrations (50 and 100 p.p.m.) killed the tissues. According to Leopold (1964) acrylic acid probably functions as a growth stimulator. There occurs strong growth promoting effect of chelating agents, fatty acids and even organic acids. The attachment of the furyl group to it has increased its activity in stimulation of growth at least in leaf tissues. Concentration of 20 p.p.m. stimulated growth during the first growth phase, which was replaced by inhibition of growth during the next phase. At 10 p.p.m. also the magnitude of stimulation declined during the
second phase culminating in clear cut inhibition during the last phase. The optimum concentration shifted laterally from 2.5 p.p.m. to 1 p.p.m. during the later two growth phases.

The lateral shifting of optimal concentration in skatole and Beta-(2-furyl) acrylic acid and deepening of inhibition at supra-optimal concentration (in auxin too) with passage of time may be interpreted as being due to a process of gradual penetration of growth regulating substances into the site of action marking it gradually more and more saturated or consumating fully the growth centres which are the focal point of growth. This results in rendering an optimal concentration a supra-optimal one, and a supra-optimal a toxic concentration which cannot be altered even by the addition of a stimulatory compound. Consequently the growth centre is lost for growth activity, the concentration being non-physiological. Physiological range of concentrations are the low range of concentrations, those below that which elicits maximum growth rate.

Perusal of stimulation by IAA revealed 0.05 p.p.m. as optimal during all the three growth phases. Growth stimulation from lower to higher concentration was quite conspicuous till it reached the optimal peak at 0.05 p.p.m. and then gradually sloped down at 0.1 and
0.5 p.p.m. with marked inhibition at 1 p.p.m. This later concentration proved to be supra-optimal which then shifted laterally to 0.5 p.p.m. during the next two growth phases. This finding goes against that of Went and Thimann (1937) who observed elongation of veins and midrib of intact leaves, and inhibition of mesophyll growth as a result of IAA application. But it is supported by Hashimoto (1959) and Dale (1966) who reported stimulation of expansion of the leaf disks obtained from Pisum and P. vulgaris respectively. The present finding is in full agreement with that of Dale (1966) in that the higher concentration of IAA induced distortion of leaf disks. IAA even at a concentration of $10^{-5}$ M was reported to inhibit growth while at $10^{-6}$ M (about 0.2 p.p.m.) marked stimulation ensued. Dale attributed this growth to cell expansion and not to cell-division. The stimulation of growth might be attributed to the softening of the cell-wall by increasing its plasticity. Such an effect of IAA was reported in Avena coleoptile growth (Heyn, 1931, 1940; Tagawa et al. 1957; Cleland, 1958; Preston and Hepton, 1960) and the increased plastic bending was reported to commensurate with the stimulatory effect on growth (Bonner, 1960). With the increased plasticity the cell-wall pressure is reduced (Ordin et al. 1956) which resulted in osmotic uptake of water and sucrose. The increased amount of sucrose
and water led to the synthesis of new cell-wall materials. This view is supported by several authors (cf. Introduction Chapter) who observed increased amount of hemicellulose and pectic substances in oat coleoptiles. Sucrose, which is a rich energy source is utilized in increased respiration as a result of IAA application. This increased rate of respiration (Galston and Purves, 1960; Nooden and Thimann, 1965; Sperling et al. 1963; Newcomb, 1960) is one of the possible causes of IAA-induced growth stimulation. This point is further clarified in the IAA-sucrose interaction studies made by the author. IAA was applied in a wide range including both stimulatory and inhibitory concentrations. The interaction surface revealed a synergistic effect between IAA and sucrose. During the first growth phase an additive effect was observed which ultimately turned out to be highly significant at the end of the third growth phase indicating a clear cut synergism between the two. With high concentration of IAA, sucrose was needed in larger amounts probably to meet the need of food and energy for cellular metabolism. With higher concentration of auxin, the lower concentration of sucrose failed to satisfy the increasing demand of food which resulted in growth depression. However, lower concentration of IAA in conjunction with lower concentration of sucrose produced synergistic effect. This
finding establishing synergism between IAA and sucrose is supported by the findings of several workers in *Avena* coleoptile section growth (Schneider, 1938; Galston and Hand, 1949; Nitsch and Nitsch, 1956; Chinoy et al. 1957; Brian and Hemming, 1958).

That protein and RNA synthesis are necessary for the auxin induced growth have been reported (Nooden, 1966; Nooden and Thimann, 1963, 1965, 1966) and that the synthesis of protein and RNA is enhanced by auxin has also been reported by these workers. Armstrong (1966) proposed that auxin may act as an initiator of certain polypeptide chains, the hormone being bound to the N-terminal of some protein. Now, it seems quite probable that IAA enhanced synthesis of RNA and protein in cells culminating in expansion of the leaf disks.

The mechanism of auxin stimulation is still a moot point. Auxin action is considered to take place in two steps. In the preparatory action stage the auxin molecule is adsorbed at the membrane (Veldstra, 1944 a, 1944 b; Veldstra and Booij, 1949), causing opening of the membrane, and thus permitting an increased flow of metabolites. Burstrom (1942) suggested that this phase corresponds to the first phase of cell elongation. After penetration into the protoplasm, the secondary phase of auxin
action begins and the active molecule exerts its primary growth action. But it seems unlikely that these actions are caused by the auxin functioning as a part of an enzyme molecule. Rather it has been established that auxin activates an enzyme system. McRae and Bonner (1953 a) in their studies of auxin kinetics demonstrated that auxin combines with an enzyme to form an intermediate complex and this complex is formed at an active site of an enzyme. The complex is converted to the enzyme and reaction products as follows:

\[ E + S \rightarrow ES \rightarrow E + P \quad \ldots \quad (1) \]

In this scheme presented by McRae and Bonner, enzyme (E) is the auxin receptor, substrate (S) is the auxin applied, complex (ES) is the receptor-auxin attachment, and the product (P) in this case is growth. Thus, auxin probably combines with enzyme to form the complex which eventually results in leaf disk expansion. Bonner and Foster (1956) too, explained auxin-induced growth promotion or inhibition in this light and stated that auxin interacts with some receptor entity of the tissue and the growth rate is proportional to the amount of complex thus formed. This concept of auxin interacting with specific reactive sites of the plant tissue for the promotion of growth and the kinetic consequences lend support to the assumption that
some sort of saturation phenomenon is involved in auxin-
induced growth promotion or inhibition.

The inhibition induced by supra-optimal concentration of IAA is explained by Skoog et al. (1942) and Foster et al. (1952) from a different point of view. They envisaged that auxin acts by attaching itself to some (enzymatic) entity in the cell. In stimulating growth it becomes attached at two positions (Two-point attachment theory). Dual requirement is necessary for auxin activity — one in aromatic ring and other in acidic side chain. If two molecules become attached at the same site, one molecule on each of the two positions, they would mutually inhibit by preventing the complete double attachment. The inhibition induced by higher concentration of IAA might be due to this saturation phenomenon.

Similarly, auxin-activity is also explained in the light of "three-point attachment" theory postulated by Smith and Wain (1952). According to this theory, an auxin molecule in order to be active must contain — an unsaturated ring, a carboxyl group and at least one alpha hydrogen. It has further been stipulated that all three must be correctly oriented in space with each other. Contact is made by the auxin-molecule with the reactive site simultaneously at three positions. If only one site or
even two positions are occupied, no activity ensues. The inhibition at supra-optimal concentration is explained as the result of incomplete attachment of auxin molecule at the reactive site. At supra-optimal concentration, probably the reactive site becomes saturated with auxin molecules which renders the attachment incomplete and they mutually inhibit their growth promoting activities.

**NMSA** at all its concentrations tried inhibited growth. Inhibition turned out to be highly significant on statistical analysis. The highest concentration (250 p.p.m.) of NMSA at the last phase of growth inhibited growth to the extent of about 15%. It is probable that both auxins and their homologous anti-auxins function competitively in the same growth centre, one compound counteracting the effects of the other. Such a mutual antagonism was reported by Åberg (1950, 1952, 1956), Audus and Das (1955) and Burstrom (1950) in root tissues. Audus (1959) holds the view that the term anti-auxin should be reserved for those compounds which compete with auxin for some specific reaction centre in the growing cell. NMSA satisfied this requirement since it suppressed the normal growth culminating in marked inhibition. It seems quite probable that NMSA knocked off the endogenous auxin from its site of action. Thus, both competed at the same growth centre resulting in competitive antagonism.
For fuller elucidation of this problem, interaction between IAA and NMSA was studied. The interaction curves depicted such mutual antagonism during all the three growth phases under observation. The stimulation caused by the stimulatory range of concentrations of IAA was completely nullified by NMSA. Such an interaction was also observed by Åberg (1950, 1953, 1955), who claimed to have succeeded in counteracting the auxin-inhibition by applying NMSA and NMSP. His findings were supported by several workers (cf. Introduction Chapter). It is quite likely that the anti-auxin competes with normal substrate of an enzyme for active sites on that enzyme. Following McRae and Bonner (1953) the formation of an enzyme-inhibitor complex may be illustrated as follows:

\[ E + I \rightarrow EI \quad \cdots \quad \cdots \quad (2) \]

The formation of EI is reversible and keeps open the competition by the substrate with the inhibitor for an active site. Therefore, by increasing the substrate (auxin), inhibition by a competitive inhibitor may be overcome. NMSA acting alone caused marked inhibition only because it super saturated the growth centre due to its higher concentration. Even the stimulatory effect of exogenous IAA was completely masked by it resulting in inhibition. The lower concentrations (10 and 50 p.p.m.) of NMSA even tended to relieve the inhibition induced by 1 p.p.m. of IAA.
But the interaction during all the three growth phases did not reach a statistically significant value. Therefore, it can be concluded that this interaction is apparent at least in leaf tissues and not real.

Within the wide range of concentrations (10-200 p.p.m.) applied, gibberellio acid stimulated growth significantly. The overall growth stimulation by this compound surpassed those by others and the magnitude of stimulation gradually increased with the progress of time. Stimulation as high as 18.27% was registered after the third growth phase at the optimal concentration of 20 p.p.m. No inhibition was induced even at the highest concentration (200 p.p.m.) of GA. This lends support to the view expressed by Leivonen (1958) that plants can stand a wider range of gibberellic acid than do the auxins and gibberellins do not appear to inhibit root growth in a manner identical to that caused by auxins. The larger growth promotion by GA is probably through its auxin-apararing action. That gibberellic acid increases the level of endogenous auxin in stem and coleoptile tissues has been established convincingly (Erygin et al. 1961; Mura-kami, 1961; Kuraishi and Muir, 1962 etc.). At lower concentration of auxin mesophyll expansion and vein elongation probably proceed concurrently at almost equal pace. The inhibition produced by higher concentration of auxin
is probably counteracted by gibberellic acid through its own effect on mesophyll expansion. This seems quite likely that GA induces growth both in the mesophyll and veins in equal proportion or if GA affects only mesophyll growth, the increased level of auxin specifically promotes growth of veins to keep pace with the mesophyll growth (Humphries and Wheeler, 1963). GA probably increases the auxin level by affecting the oxidation of IAA by IAA-oxidase enzyme system. GA is also reported to cause accelerated conversion of auxin precursor into free available auxin (Bednarz et al. 1967). Moreover, GA-induced growth is influenced largely by sucrose present in the medium. An accelerated growth stimulation in roots was reported by Butcher and Street (1960) up to 1% sucrose in the medium. Sucrose seems to have played similar role in the expansion growth of bean leaf disks in conjunction with GA. Thus, these factors, viz. GA, IAA and sucrose have had their combined additive effect which resulted in largest leaf disk expansion as compared to other factors. To have a better idea of such synergistic effect of GA and IAA the problem was studied by allowing the leaf disks to grow in media containing the combination of both the compounds. The broad pattern of growth revealed well-marked synergism. The interaction between IAA and GA did not reach the significant level during the first growth phase.
But, during the next two growth phases interaction turned out to be highly significant indicating positive response of the leaf tissue to added GA and IAA. This is in conformity with that of Gorter (1961) with pea stem sections. Synergistic effect between IAA and GA was also reported by several workers in different kinds of tissues (Galston and Mc.Cune, 1961; Brian, 1958; Galston and Warburg, 1959). The present findings revealed that GA induced stimulation at IAA concentration of 0.1 p.p.m., which caused growth depression. During all the three phases under observation it was found that GA did not promote growth where IAA (1 p.p.m.) induced inhibition, but it appears that it tended to reverse inhibition which resulted in prevention of curling of the leaf disks. This result is substantiated by the report made by Kato (1958) experimenting with pea stem sections. This observed synergism between IAA and GA might be due to the GA effect which somehow increases the availability of sites for auxin to function more efficiently.

GA is also considered to be a cell-division factor which is manifested in the elongation of sections (Greulach and Haasloop, 1958; Sachs et al. 1957, 1959, 1960). Cell-enlargement and cell-division as the after-effects of GA treatment have been established (cf. Introduction Chapter) by several researchers. Cell-division
might be one of the probable causes of largest stimulation of leaf disk expansion. Dale (1966) attributed the increase in fresh weight and dry weight of Pisum vulgaris leaf disks to cell-division brought about by GA (10 mg/l). The leaf expansion may also result from increased level of invertase as a result of GA application as evidenced in other tissues (Kaufman, 1965; Kaufman et al. 1968; Hayashi et al. 1964; Paleg, 1960; Varner, 1964; Varner et al. 1964). This probably resulted in releasing reducing sugars that was used in polysaccharide biosynthesis in expanding cells.

Bora and Selman (1969) suggested that GA by stimulating RNA and protein synthesis, increases the production of native gibberellins, and it is through their action that growth is accelerated. It can thus be concluded that GA-induced leaf disk expansion is the result of manifold activities. It seems quite likely, that GA accelerated the production of endogenous auxin and native gibberellin synchronously with the synthesis of proteins and RNA. The largest disk expansion as had been observed in this investigation might be the result of combined action of auxin and GA which was further enhanced by suitable concentration of sucrose in the media. That stimulation gradually increased with the progress of time might have resulted due to renewed activity by cell-division.
Kinetin caused pronounced growth stimulation right from 0.01 p.p.m. to 50 p.p.m., 10 p.p.m. recording as optimal. Stimulation as high as 12.02% over the corresponding control was registered after the third growth phase at the optimal concentration. The promotion of growth stimulation increased with the progress of time. This finding of bean leaf disk expansion as a result of kinetin application is supported by Miller (1956), Scott and Liverman (1956), Powell and Griffith (1960) and Humphries and Wheeler (1960). Leaf disk expansion is considered to be the result of cell-expansion rather than cell-division (Miller, 1956; Kuraishi and Okumura, 1956). On the other hand, kinetin has unequivocally been established as a cell-division factor. Kinetin in combination with IAA is reported to maintain continuous increased growth stimulation in various kinds of tissues (Miller et al. 1956; Skoog et al. 1957; Levee and Messer, 1969). IAA and kinetin when applied alone, stimulate DNA synthesis (Das et al. 1956). Both the compounds are needed to bring about cell-division, but IAA seems to dominate by bringing about duplication of DNA in mitosis, whereas kinetin causes cytokinesis. The adenine moiety of kinetin molecule appears essential for mitosis, many different substituted side-chains being applicable. It is probable that the side-chain may influence some physical property (such
as solubility) with a bearing on the efficiency of the growth regulator to induce cell-division. Whether this observed leaf disk expansion is the result of cell-division associated with cell-expansion or only the result of cell expansion was studied during the course of present investigation by applying stimulatory concentrations of kinetin in conjunction with stimulatory and inhibitory concentrations of IAA. A larger growth promotion was observed during all the three growth phases. But the interaction between the two factors did not turn out to be significant. This larger growth promotion was an additive effect and not a case of synergism (more than additive). The optimum concentration of IAA (0.05 p.p.m.) shifted laterally to 0.01 p.p.m. and the inhibition induced by IAA was also relieved by kinetin during all the three growth phases. Kinetin probably caused mobilization of sucrose and auxin from the ambient solution. Mothes et al. (1959), Mothes (1960) and Richmond and Lang (1957) observed mobilization of nutrients and synthesis of protein and chlorophyll in kinetin treated area of intact leaves. In the present findings, kinetin when applied alone caused mobilization of sucrose resulting in larger growth stimulation. When IAA was present in the medium, both IAA and sucrose probably made rapid entry into the cells. This might have caused shifting of optimal
concentration of IAA to 0.01 p.p.m. On the other hand, inhibition at higher concentration was also relieved due to mobilization of sucrose. The higher concentration of IAA in response to increased concentration of sucrose caused marked stimulation of growth. Such results are already discussed in IAA-sucrose interaction studies made by the author (Chapter VIII). The possibility of induction of cell-division as a result of kinetin application cannot be ruled out outright. The increasing rate of stimulation suggests such combined effects of IAA and kinetin. Another reason which supports their additive effect might be their influence on two different sets of mechanism. IAA causes elongation of the veins more rapidly (Went and Thimann, 1937) while kinetin is responsible for larger expansion of mesophylls without affecting the veins (Miller, 1956). When both the factors were combined, probably resulted in greater expansion of leaf disks.

There are reports (Guttman, 1956; 1957) of increased level of RNA as a result of kinetin application. Thimann and Laloraya (1960) held the view that the influence of purine on RNA might be responsible for its ability to protein synthesis. Kinetin when applied to the plant tissue, perhaps become fixed into the complex compound such as RNA. This would be consistent with the known effects
of kinetin on RNA and protein synthesis and would also account for the high degree of localization of kinetin effects (Mothes et al. 1959; Thimann and Laloraya, 1960; Mueller, 1964; Partheir et al. 1961). The ability of kinetin to induce synthesis of vitamins (Dravniecks et al. 1969; Bergmann and Bergmann, 1968; Digby and Skoog, 1966) has also added to its activity to the expansion of bean leaf disks.

Increased rate of respiration might be one of the probable causes of disk expansion. Increased respiration might have resulted in rapid uptake and breakdown of sugar in consequence of which more energy was liberated. Glasziou (1957) reported such a phenomenon in tobacco pith slices.