CHAPTER V
This investigation was carried out mainly to have fuller appraisal of the effects of plant growth regulators (phyto-hormones, inhibitors, anti-auxins and growth retardants) and their interactions on 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 (particularly sugar) necessary for growth and development as a result 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 for food. It was expected that this sort of experimentation would help to give a unified picture of the requirement of exogenous food factors and help us in understanding the exact role played by the wide range of plant growth regulators in the physiology of leaf expansion.

Altogether three carbohydrates viz. glucose, fructose and sucrose were tried with the same range of concentrations (0.5, 1, 2, 5 and 10%). All of them exhibited highly significant stimulatory effect. Stimulation increased gradually from 0.5 to 5% (optimum) and declined at 10%. The stimulation at the optimal concentration of sucrose was measured as 17.20, 36.24 and 45.50% over the initial diameter after 24, 48 and 72 hr respectively. Stimulation at the optimal concentration of glucose was recorded as 15.99, 25.19 and 33.09% while that of fructose was measured as 10.48, 18.08 and
24.55% over initial diameter after 24, 48 and 72 hr respectively.

This finding well illustrates the imperative need of carbohydrate in the culture media as an energy source for the sustained growth of tissues. This lends support to the view expressed by Gautheret (1955) and Sarma (1971) that the plant tissue must be cultured in media supplemented with sugar. The tissues do not survive for long in cultures in the absence of an external supply of carbohydrates (Hildebrandt et al. 1963, Edelman and Hanson 1971, 1972; Hanson and Edelman 1972, Pamplin and Chapman 1975).

In leaf tissue cultures in recent years, glucose or sucrose has found wide application in constituting the basal medium (Butenko et al. 1972, Mathes et al. 1973). Over many years glucose (Gautheret 1942) or sucrose (White 1943) has been empirically used in concentrations ranging from 2 to 5%. Miller (1951, 1952) used 3% D-glucose in the basal medium for rearing up leaf disks isolated from etiolated bean seedlings. In a later experiment, Miller (1956) supplemented the basal medium with about 2% (5.8 x 10^{-2}M) sucrose. Sarma (1971) observed well-marked optima in the range of 0.5 to 5%.

In relative efficiency of all carbohydrates tried, sucrose proved superior to all other, followed by glucose and fructose. This is in conformity with the findings of Gautheret (1945), Hildebrandt and Riker (1953) and Henderson (1954). Sucrose or one of its monosaccharide components proved indispensable for maintaining sustained growth and tissue differentiation (Lyne and apRees 1971, Moore et al. 1972, Adams et al. 1973, Weston 1973, Parr 1974, Parr et al. 1976).

The osmotic pressure of the solution apparently control the rate of absorption of sugars into plant cells. Examination of growth
rate during three intervals of time reveals that the magnitude of stimulation gradually declined from the first to the third period. For example, glucose (5 %) exhibited 15.99, 9.20 and 7.90 % stimulation after 24, 48 and 72 hr respectively. This trend was with fructose and sucrose too. This growth pattern can be attributed to the rapid uptake and utilization of sugars during the first growth phase (0-24 hr). The efficiency of absorption and utilization perhaps fall with the aging of leaf tissues coupled with depletion of endogenous growth factors. Higher concentration of sugars (10 %) probably acted as hypertonic solution exhibiting growth decline and inhibition.

Indole-3-acetic acid : IAA stimulated growth within the range of concentrations (0.01 to 1 ppm) tried. The growth response curves (Fig 4) indicate that the stimulation elicited by IAA gradually increased from 0.01 to 0.5 ppm during all the periods under observation and then declined at 1 ppm. The stimulation caused by optimal concentration (i.e. 0.5 ppm) of IAA was recorded as 19.27, 25.58 and 30.31 % after 24, 48 and 72 hr of incubation. This finding is in agreement with those of Hashimoto(1959), Dale(1966) and Sarna(1971) who reported stimulation of expansion of the leaf disks obtained from Pisum and Phaseolus vulgaris by I.A. The present finding substantiates those of Dale(1966) and Sarna(1971) in that the higher concentration of IAA caused distortion of leaf disks. Dale(1966) attributed this growth promotion to cell expansion and not to cell division. Expansion of leaf disks gradually increased from the first period to last period under observation.
The stimulation of expansion growth during the first period may be attributed to the auxin action as proposed by Hager et al. (1971) and substantiated by several other workers (Evans et al. 1971, Rayle and Cleland 1972, Rayle 1973, Farre et al. 1975, Nelles 1977). Hager et al. (1971) proposed that auxin stimulates an outwardly directed H⁺ pump (proton extrusion pump), thereby acidifying the cell-wall and lowering the pH of the medium with consequent loosening and expansion of cells. Several workers reported that auxin induced a pH drop in the solutions bathing treated tissues (Lado et al. 1972, Yamamoto et al. 1974, Cleland 1975) and others have documented the acid growth effect and lent indirect support to an active acidification mechanism (Durand and Rayle 1973). The primary effect of added auxin may be due to lowering of the pH of the medium that permeates the cell-walls resulting in expansion of the leaf disks. The primary action of auxin is not dependent on new protein synthesis (Rope and Black 1972). Such primary rapid action of auxin was observed in pea stem segments within 15 minutes of hormone treatment (Abdul-baki and Ray 1971). Identical rapid stimulatory effect of IAA was also reported in wheat coleoptile section by Schlienger et al. (1977). Within 1 hr of treatment with IAA 2- to 4-fold increase in the particulate UDP glucose-1,4-β glucan gluconsyltransferase activity in pea stems was reported (Ray 1973). This increase is reported to be the result of enzyme activation rather than enzyme synthesis. That IAA induced cell-wall loosening is caused by the degradation of non-cellulosic β-glucan by enzyme β-1,3-glucanase (Fujimoto and Hasuda 1968, Wada et al. 1968) in the cell wall has also been reported by Sakurai et al.
In the present finding it is found that the leaf disks maintained a sustained growth response to IAA. Varner and KO (1976) envisaged that a sustained response of a growing tissue to IAA would require concomitant protein synthesis and RNA synthesis. Wooden and Thirann (1963) suggested that auxin stimulates growth by regulating the synthesis of some growth-limiting protein via control of mRNA synthesis which was supported by Trewavas (1968), O'Brien et al. (1968) and Key (1969). Besides, the expansion of leaf disks elicited by added IAA may be due to changes in plasticity (Bennet 1960) and elasticity (Fasuda 1966) of cells, synthesis of new cell wall materials such as cellulose and hemicellulose (Christiansen and Thirann 1950) and pectins (Koore and Olson 1965). The increased plasticity of the cell-wall augments the water potential gradient towards the interior of the cell, since water entry has previously been restrained by wall pressure (Galston & Davies 1970). With the increased plasticity the cell-wall pressure is reduced (Ordin et al. 1956) which resulted in osmotic uptake of water and sucrose (Harada & Katsumi 1978). Plasticity is a non-reversible wall deformation that probably is caused by breaking of crosslinks between the cellulose microfibrils of the cell wall (Galston & Davies 1969). The increase in cell size occurs in two stages: (1) loosening of the cell wall followed by uptake of water and an expansion of the wall. Enlargement of the plant cells is regarded in principle as a result of vacuolation and accompanying water uptake of the cell. The latent potential of the water uptake is primarily determined by the osmotic potential of the cell. However the actual degree of water uptake is
limited by the rigidity of the cell wall (pressure potential). Auxin loosens the cell wall to lower the pressure potential, thus allowing more water uptake. Then increased water uptake maintains a swelling force against the softening cell wall (Bonner et al. 1953) resulting in cell enlargement. Thus, auxin through its manifold activities promotes growth stimulation immediately after its addition to the growth media. It also helps tissues maintain sustained growth.

The stimulation of growth declined at 1 pM of IAA. During all the periods of growth under observation the magnitude of stimulation remained almost constant at this concentration. The growth declined at the higher (supra-optimal) concentrations of IAA can be attributed to saturation phenomenon which was explained by Skoog et al. (1942) and Foster et al. (1952). They envisaged that auxin acts by attaching itself to some entity (enzymic) in the cell. When the attachment is complete at two positions ('Two-point attachment Theory') or three positions ('Three-point attachment Theory' of Smith and Wain 1952) auxin exerts its stimulatory actions. But at higher concentrations two or three molecules get attached at the same site making the attachment of auxin molecules incomplete and growth inhibition ensues. Purp and Purp (1964) envisaged that auxin at or near the optimum concentration for growth, begins to induce the formation of ethylene and with successively increasing levels of auxin beyond this point, the tissue is progressively inhibited by the increased levels of ethylene formed.
Gibberellic acid: In the present investigation $GA_3$ was applied at the concentrations of 1, 10, 20, 50 and 100 ppm. $GA_3$ evoked high degree of stimulation of expansion of the leaf disks of bean. The magnitude of stimulation gradually increased with the progress of time. The stimulation gradually increased from 1 to 20 ppm and then declined at 50 and 100 ppm of $GA_3$. At the optimal concentration (20 ppm) the stimulation was recorded as 16.83, 36.34 and 46.71% after first, second and third growth period respectively. No inhibition was induced even at the highest concentration (100 ppm) of $GA_3$ applied. This lends support to the view expressed by Leivonen (1958) that plants can stand a wider range of gibberellic acid. Promotion of leaf disk expansion by $GA_3$ was also reported by several workers (Dale 1966, Sarma 1971, Jones 1973).


Gibberellin frequently increases auxin content by stimulating the synthesis of polyhydroxycinnamic acid (Kögl & Elema 1960) and the latter compound in turn inhibits IAA-oxidase, thus promoting auxin-mediated process in plants by reducing the amount of auxin destroyed by enzyme. There is another suggestion that gibberellin plays a more direct role in stimulating the formation of auxin.
Treatment of tissues with GA$_3$ induces the formation of proteolytic enzymes that would be expected to release tryptophan, a precursor of IAA (van Overbeek 1966). Then GA$_3$ stimulates the conversion of tryptophan to IAA (Nair and Lenticar 1969). The disk expansion through vein elongation may be favoured by the increased auxin level coupled with mesophyll expansion by GA$_3$ itself. Humphries and Wheeler (1963) envisaged that Gibberellic acid induces expansion of the mesophyll cells without affecting vein elongation. On the other hand auxin was considered to be a potent growth factor affecting vein elongation without its effect on mesophyll cells (Kent and Thimann 1937). The combined effect might have resulted in marked stimulation of growth. However, the presence of sucrose in the medium might have accelerated the growth as suggested by Purves and Hillman (1958). GA$_3$-mediated growth promotion might be through cell division evoked by gibberellic acid and cell expansion caused by auxin (Humphries and French 1963, Dale 1966). This observation of growth stimulation of leaf disks is supported by the results of Scott and Livermann (1956), Humphries and Wheeler (1966), Dale (1966), Jones (1973), Low (1975). Gibberellic acid may also promote cell expansion by affecting osmotic pressure of the cell-sap. The gibberellin-induced alpha-amylase (Filner and Varner 1967) might increase the concentration of sugar by hydrolysing the starch which increase the osmotic pressure of the cell-sap so that water enters the cell and helps stretching the cell-wall already weakened by enzymes (Weaver 1972). GA$_3$ stimulation of expansion appears to involve an early attraction in the
formation of some membrane components of the cell such as endoplasmic reticulum (Jones 1960). By using radio-active isotopes Montague and Ikuma (1975) observed the stimulation of incorporation of $^{14}C$ glucose into cell-wall synthesis after $GA_3$ application. Permeability of cell-wall is strikingly increased upon addition of GA (Wood and Paleg 1972). Further it is suggested that gibberellin modifies the RNA synthesis through the stimulation of DNA synthesis (Jarvis et al. 1968) which in turn synthesizes new enzymes (Grieshaber and Fellenberg 1971).

**Cytokinins** : Within the wide range of concentrations (1, 10, 20, 50 and 100 ppm) applied, kinetin stimulated expansion growth of leaf disks significantly. At the optimal concentration (20 ppm) the stimulation was recorded as 3.19, 6.98 and 13.49 % after 24, 48 and 72 hr of incubation. 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 in conformity with those of Powell and Griffith (1966), Humphries and Wheeler (1966), Sarna (1971). Kinetin induced growth stimulation of leaf tissues is explained as arising from cell expansion rather than its known activity on cell division (Went 1951). Provine and Farber (1966) reported an influence on microfibril orientation in the cell wall as a basis of the cytokinin effect on cell enlargement. Although the influence of these phytohormones has been investigated on DNA, RNA and protein metabolism, a general understanding of their molecular mode of action has not yet emerged (Skoog and Armstrong 1970, Kende 1971, Hall 1973, Burrow 1975, Shinninger and Polley 1977, Roer and Feierabend 1978).
In leaves cytokinins also strongly affect the accumulation and retention of metabolites (Lothee 1960, Kende 1971). They observed mobilization of nutrients and synthesis of protein and chlorophyll in kinetin treated area of intact leaves. These results conform with those of Richmond and Lang (1957), Müller and Leopold (1966) who observed mobilization of nutrients to the treated area. The movement of the materials involves not only phloem transport (Kursanov 1963) but in leaves occurs cell to cell movement. Such transport may be due in part to an influence on the formation of proteins or other large molecular aggregates but one principal mechanism of cytokinins apparently involves transport against concentration gradient (Skoog and Schmitz 1972). In the present finding cytokinins probably caused mobilization of sucrose and nutrients from the ambient solution resulting in large growth stimulation.

Kinetin is reported to cause marked increase not only in RNA but DNA and protein synthesis as well (Farthier and Wollciehn 1961) in tobacco leaves, which indicates that in the delaying of senescence kinetin acts on the overall synthetic activities of leaf. Kinetin is reported to increase DNA synthesis in cultures of disks of potato and stimulate glucose catabolism by the pentose phosphate pathway (Kikune et al. 1977). Kinetin also stimulates the rate of RNA synthesis prior to any known response (Shininger and Polley 1977), the nuclear RNA synthesis and regulates the release of RNA into cytoplasm (Roy Choudhury et al. 1965). Besides, kinetin induces an increase in the amount of endoplasmic reticulum and cytoplasmic ribosomes (Shaw and Filoncic 1965). Kinetin is also reported to delay
the senescence of detached leaves by slowing down the process of protein degradation (Richmond and Lang 1957, Mizrahi et al. 1970). This finding was substantiated by the findings of several other workers (Rende 1971, Varga and Bruinsma 1973, Wang 1977). While preserving the protein content of detached leaves (Shibaoka and Thimann 1970), kinetin also enhances the synthesis of new protein (Prady et al. 1971), Maass and Klambt 1977). It is also reported to stimulate the synthesis of starch in detached leaves (Mittelheuser and van Steveninck 1972).

Cytokinin treatment increases bound auxin (Hemberg 1972). Jordon and Skoog (1971) and Skoog and Armstrong (1970) also envisaged that cytokinin brings about an increase in endogenous auxin. Such a view finds support from the findings of Largerstedt and Langston (1967) and Hemberg and Larsson (1972) who showed that in plant parts or leaves treated with kinetin an accumulation of endogenous auxin takes place in the treated area. Under the influence of lower concentration of kinetin probably lower amount of auxin accumulated resulting in growth stimulation. At higher concentration (i.e. 100 ppm) there was growth inhibition, probably due to accumulation of supra-optimal concentration of auxin.

Benzyladenine was also tried with the same range of concentrations (1-100 ppm) as kinetin. Stimulation turned out to be highly significant on statistical analysis. Perusal of stimulation by BA revealed 50 ppm as optimal during all the three growth phases. Growth stimulation increased gradually from 1 to 50 ppm (optimal concentration) and resulted in marked inhibition at 100 ppm. The stimulation elicited by optimal concentration of BA was recorded as 16.02, 24.33,
and 41.18% after 24, 48 and 72 hr of incubation. The inhibition caused by 100 ppm was gradually alleviated with the progress of time recording 9.17 and 0.46% inhibition after 24 and 48 hr and exhibited 1.43% stimulation after 72 hr. The overall growth stimulation by BA was higher than that elicited by kinetin, but the growth response at 100 ppm was almost identical with that of kinetin. Leaf disk expansion by BA was also reported by Handro et al. (1977) and Berridge et al. (1970).

Synthetic cytokinins BA and kinetin delayed senescence (Wang 1977) and increased transpiration (Biddington and Thomas 1978) of leaves. Cytokinins delay senescence in some excised leaf tissue by maintaining chlorophyll, nucleic acids and proteins at higher levels than in untreated tissue (Richmond and Lang 1957, Mizrahi et al. 1970, Kende 1971).

That kinetin and BA act as antisenescence agents and enhance chlorophyll synthesis has also been reported by several other investigators (Bant rji and Laloraya 1967, Fletcher and McCullagh 1971, Fletcher et al. 1973). They also observed increase in chlorophyll content of growing cotyledonary leaves as a result of cytokinin application.

Berridge et al. (1970) reported that stimulation of expansion growth of Chinese cabbage leaf disks by 6-BA and kinetin may be due to direct effect on protein synthesis by attaching to cytoplasmic ribosomes. It has been suggested that BA-induced growth stimulation is mediated through protein synthesis. In leaf disk cultures, Tsibulya (1977) and Tsibulya and Kulaeva (1977) observed that 6-BA stimulated
leaf disk growth in conditions of undisturbed protein synthesis but addition of cycloheximide or Actinomycin D which inhibits protein synthesis or RNA synthesis, the stimulation effect of 6-BA was completely blocked.

Thus the stimulation of expansion growth of leaf disks appear to have induced through synthesis of protein, delaying the senescence of leaf disk and through mobilization of sucrose and nutrients from the ambient solution.

They also increase stomatal aperture and hence increase transpiration (Livnè and Vaadia 1965). Raschke (1975) has suggested that the action of cytokinins or transpiration is indirect and may be related to their effects on senescence. Recent work by Kuraishi (1976) indicates that cytokinin-induced chlorophyll retention may be mediated through the hormones' effect on stomatal opening. Das et al. (1976) observed that whereas kinetin had no remarkable effect on stomatal opening, the activity of BA was observed over a narrow range of concentrations.

Coumarin, a potent growth inhibitor was found to stimulate expansion growth of leaf disks. Coumarin was applied at 10, 20, 50 and 100 ppm. All the concentrations except 100 ppm proved stimulatory and 50 ppm stood as optimal during all the three periods under observation. At the optimal concentration (50 ppm) the stimulation was recorded as 7.90, 12.02 and 14.75% after 24, 48 and 72 hr of incubation. This finding substantiates those of Miller and Mayer (1950-51) and Sarma (1971) who reported similar growth promotion of Chenopodium album and bean leaf disks, using a wide range of concentrations of coumarin. Stimulation of growth by low concentrations
(Neumann 1959, Mayer and Poljakoff Mayber 1961) and high concentrations (Neumann 1960, Knypl 1964 a,c) has also been reported.

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, synthesis of RNA, proteins and ATP (Knypl 1964, 1965). He further observed increased respiration of Phaseolus vulgaris leaf tissues as a result of coumarin application. Protection against or control of IAA-oxidation by inhibiting the activity of indole-acetic oxidase might be envisioned as having a regulatory role in plant growth (Miller et al. 1975). Thus, the stimulation of growth 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.

Recent studies have established that coumarin stimulates the cell expansion and increases the elasticity of growing cell walls both in shoots and roots (Svensson 1971, Uhrström and Svensson 1979). It seems likely that the structure of the cell wall or plasmalemma is affected by coumarin (Svensson 1971) and that a change in pattern of deposition of cell wall matter has taken place (Albersheim et al. 1977, Burström 1975). Thus, by increasing the elastic tensility, cell walls are made more permeable to water and nutrients as the auxin does (Lado et al. 1976). This may be another factor contributing for cell expansion in bean leaf disks.
Abscisic Acid

In the present investigation Abscisic acid (ABA) was applied at the concentrations of 0.1, 1, 10, 20 and 50 ppm. All the concentrations proved highly inhibitory to leaf disk growth. The magnitude of inhibition gradually increased with the increasing concentrations and with the progress of time. Inhibition at 50 ppm was recorded as 16.31, 20.52 and 25.15 % after 24, 48 and 72 hr respectively.

Working with leaf disks, Aspinall et al. (1967), Pack and Richmond (1971), De Leo and Sacher (1970) and several other investigators reported that ABA acts as a senescence agent. ABA enhances the degradation of chlorophyll which can be prevented by additions of kinetin or benzyladenine (Aspinall et al. 1967, Pack and Richmond 1971) and by potassium ions (Sanblija 1971). Senescence of radish leaf disks was promoted by ABA within the first 24 hr of excision (Colquhoun and Hillman 1972), but when applied after 6-day incubation period, ABA retarded the decline in pigment content although it promoted the decline in protein content. Evidence that the synthesis of degradative enzymes occurs in senescing leaf disks was provided by Knypl and Mazurczyk (1971) using radish leaf disks; cycloheximide and vanillin decreased the loss of protein and retarded the loss of chlorophyll while (\(\text{\textsuperscript{14}}\)) ABA increased both. ABA treatment is reported to increase the activities of phosphatase and phosphatase in Rhoco leaf sections (De Leo and Sacher 1970). Paranjethy and Waring (1971) observed that ABA promoted the senescence of leaf disks but had no effect on the incorporation of (\(\text{\textsuperscript{3}}\))H] cytidine.

Thus, the inhibition of growth by ABA can be attributed to its
primary effect on inhibition of RNA biosynthesis (van Overbeek et al. 1967, Stewart and Smith 1972, Bilet and Boera Przybecka 1975). ABA may also exert its action by inhibiting the synthesis of enzyme-specific RNA molecules or by preventing their incorporation in an active enzyme-synthesizing unit (Galston and Davies 1970). Inhibition of RNA synthesis has been reported in *Taraxacum officinale* leaves (Warring et al. 1967) and in *Lamia* (van Overbeek et al. 1967). In the present finding the inhibition of leaf disk expansion may be due to inhibition of DNA, RNA and protein synthesis. ABA is known to reverse the GA-enhanced synthesis of enzymes, membrane-bound polysome formation, and RNA synthesis (Yomo and Varner 1971, Varner and De 1976). It is also reported to prevent the auxin-enhanced cell elongation in *Avena* coleoptiles (Kahn and Cline 1973). The inhibition caused by ABA may also be due to such counteracting effects on endogenous stimulator hormones.

Maleic hydrazide was found to inhibit the growth of leaf disks of *bean*. Significant growth inhibition was found within the range of concentrations tried. MH was applied at the concentrations of 10, 20, 50, 100 and 250 ppm and all the concentrations inhibited the growth. The magnitude of inhibition increased with the rise in concentrations. But with the passage of time the intensity of inhibition gradually decreased. Thus, at the highest concentration used (250 ppm) inhibition was recorded as 12.40, 8.96 and 7.40 % after 24, 48 and 72 hr of incubation. This finding substantiates those of Sama and Borah (1973) and Sama and Phukan (1974) who observed inhibition of extension of hypocotyl segments and expansion of leaf disks of bean.
by I H. Baskakov and Butenko (1961) also observed that 5 -10^{-3} \text{g/l} \text{NH} \text{Cl} was sufficient for inhibiting the growth of an isolated callus tissue of carrot. The inhibition caused by \text{NH} \text{Cl} can be attributed to its amitotic behaviour which eventually causes growth retardation (Pilet 1956). The inhibition caused by \text{HH} may also be due to the blocking of biosynthesis of nucleic acids (Baskakov and Butenko 1961, Brian and Hemming 1957) and blocking of biosynthesis of GA (Brian and Hemming 1957). \text{NH} \text{Cl} is also reported to adversely affect the formation of, transport or utilization of auxins (Webb 1966). It was also postulated that \text{NH} \text{Cl} might produce some of its growth inhibition through the destruction of auxin by indole-acetic oxidase (Baker 1961). That the leaf disks could gradually reduce the inhibition is also supported by the findings of Creed (1975) and Haeberer et al. (1978). They observed that during active period of growth \text{NH} \text{Cl} content declined with time.

**Tri-iodobenzoic acid (TIBA)** was applied within a wider range of concentrations from 1 to 50 ppm to examine its effect on the leaf disks of *Phaseolus vulgaris*. All the concentrations evoked high degree of inhibition of expansion growth of leaf disks. The magnitude of inhibition increased with the rise in concentrations and with the passage of time. At 50 ppm the inhibition was measured as 6.11, 19.09 and 22.70 % after 24, 48 and 72 hr of incubation.

Inhibition of growth of stem and distortion of leaves as a result of TIBA application were reported by Sengupta and Chattopadhyay (1954) and Venkataratnam and Satyanarayamurthy (1950). TIBA is known to prevent the physiological actions of IAA (Venkataratnam
and Satyanarayanmurthy 1950, Kuse 1953). In tissue cultures TIOA prevents the establishment of an auxin gradient by blocking cell to cell movement and polar transport (Niedergang-Kaměln and Skoog 1956). This has been substantiated recently by Lancaster and Rowan (1971) in cultures of carrot root cambium. It appears that auxin and TIOA were mutually antagonistic one counteracting the effect of the other. Application of exogenous TIOA might have completely masked the endogenous auxin, preventing thereby the stimulatory effect of auxin. Such mutual antagonism was also reported by Åberg (1956).

**Growth retardants:**

(2-chloroethyl)trimethylammonium chloride (CCC), N,N-dimethyl-succinamic acid (N-995) and Tributyl-2,4-dichlorobenzyphosphonium chloride (Phosfon D) were used for experimentation. All the compounds were applied at higher concentrations.

CCC was applied at the concentrations of 100, 250, 500 and 1000 ppm. All the concentrations were highly inhibitory. The magnitude of inhibition gradually increased with the rise in concentration and also with the progress of time. Thus, at the highest concentration of CCC applied, the inhibition was 10.40, 14.02 and 19.87 % after 24, 48 and 72 hr respectively.

Phosfon D was tried with 3 concentrations viz. 100, 250 and 500 ppm. Identical results as in CCC were obtained. After 72 hr of incubation, the inhibition at 500 ppm was found to be 32 %. The magnitude of inhibition with Phosfon D was higher than that of CCC.

E-995 (Alar) was applied at the concentrations of 100, 250, 500 and 1000 ppm. All the concentrations proved highly inhibitory.
Thus, at the highest concentration tried (1000 ppm) the inhibition was recorded as 16.18, 29.08 and 30.37 % after 24, 48 and 72 hr of incubation.

All the three compounds imparted highly inhibitory effect on leaf disk expansion. On statistical analysis, the effects of CCC, Phosfon D and B-995 emerged as highly significant. That CCC and B-995 inhibit plant growth has been reported by several investigators. (Ninneman et al. 1964, Wilde and Edgerton 1969, Russell and Kimmins 1972, Dunberg and Eliasson 1972). Growth retardation by phosphoniums (Preston and Link 1958) and particularly by Phosfon D (Knypl 1970', Cathey and Stuart 1961) was also reported. In cultures of bean hypocotyl segments CCC inhibited extension growth but addition of GA₃ could reverse the inhibitory effects of the lower concentrations of CCC (Sarma and Deka 1977a). The growth inhibition by these compounds is considered due to inhibition of GA biosynthesis (Zeevaart 1966, Ockerse 1970), by blocking the production of kaurene with the result that geranylgeraniol accumulates (Lang 1970). But, it is also suggested that the growth inhibition may accrue from the inhibition of some other aspects of metabolism not directly related to GA's (Baldev et al. 1965, Berry and Smith 1970). Reid and Crozier' (1972) suggested that in addition to any effects on GA biosynthesis per se, retardants may inhibit GA action.

**IAA-Kinetin interactions :**

Both kinetin and IAA as potent growth stimulators inducing marked growth stimulation of bean leaf disks. Kinetin was applied at the concentrations of 10, 20, 50 and 100 ppm while IAA was applied
at much lower concentrations (0.01, 0.05, 0.5 and 1 ppm). At the optimal concentration of kinetin (20 ppm) stimulation was recorded as 13.83, 19.18 and 28.08 % after 24, 48 and 72 hr respectively. Similarly at the optimal concentration of IAA (0.5 p.m) stimulation was of the order of 12.78, 19.37 and 31.99 % after 24, 48 and 72 hr respectively. In most of the combinations during all the periods of growth. Stimulation was more than additive (synergistic). On statistical analysis, the interaction between the two factors turned out to be highly significant. The slight inhibition caused by 100 ppm of kinetin during the first two growth periods was also alleviated by addition of IAA and resulted in marked growth stimulation.

This finding is in agreement with those of Miller et al. (1955), Skoog and Miller (1957), Shrank (1956), Denizci (1966), Hemberg and Larsson (1972) and Sarma (1971) who reported that kinetin strongly enhanced the stimulatory effect of IAA on the growth of cultured tobacco wound callus culture, Avena coleoptile segments and etiolated leaf disks of bean.

Cytokinins are intimately involved in retarding senescence and in synthesis of nucleic acids and proteins (Richmond and Lang 1957, et al. Mothes 1961, Wollgiehn 1961, Osborne 1962). Cytokinins are also involved in some basic processes of cell division, expansion and differentiation (Skoog and Schmitz 1972). It was recognized that both auxin and cytokinin are required for continuous in vitro growth and cell division in tobacco tissue (Das et al. 1956, Patau et al. 1957). But leaf disk expansion is explained as arising out of only cell expansion rather than its known activity on cell division (Miller...

The expansion of leaf disks elicited by IAA may be due to loosening of the cell through an active acidification mechanism (Hager et al. 1971, Durand and Rayle 1973) activation or synthesis of enzymes, also protein and RNA synthesis (Varner and Ho 1976), Nooden and Thimann 1963, O'Brien et al. 1968, Key 1969) and rapid water uptake (Bonner et al. 1953, Galston and Davies 1970).

Thus, both the stimulators through their individual mechanism of cell expansion or combined effect on DNA and RNA synthesis and protein synthesis brought larger expansion of leaf disks. Kinetin causes a strongly localised mobilization of nutrients to the treated area. It may be that the demand for more nutrients and food was met by kinetin from the ambient solution. Such synergism between IAA and kinetin has also been reported by Butenko (1968), Adamson (1962) and Setterfield (1963).

**IAA-TIBA interactions**:

For clearer appraisal of the problem, interaction between IAA and TIBA was investigated. IAA was applied at the concentrations of 0.01, 0.05, 0.5 and 1 ppm while TIBA was applied at 1, 10, 20, and 50 ppm. IAA proved highly stimulatory recording 9.76, 25.89 and 29.22% increase over the control at the optimal concentration (0.5 ppm) after 24, 48 and 72 hr of incubation. On the otherhand, all the concentrations of TIBA proved highly inhibitory recording 18.21% inhibition at 50 ppm after 72 hr of incubation. The stimulation
elicited by IAA was reduced by TIBA and at certain combinations it resulted in marked inhibition. This can also be interpreted as alleviation of inhibition induced by TIBA. After 24, 48 and 72 hr in almost all the combinations (except 50 ppm of TIBA), there was growth stimulation. However, the overall stimulation was lower than IAA acting alone. This shows that both IAA and TIBA are mutually antagonistic.

This finding is in conformity with that of Galston (1947) who reported that TIBA causes suppression of auxin content. This was substantiated by Audus and Thresh (1956). The stimulation of growth elicited by IAA was found to be reduced by TIBA and the inhibition caused by TIBA was also alleviated by IAA. This establishes the counteracting effect of IAA and growth suppressor (Corcoran 1975). TIBA acting alone was found to inhibit growth at all its concentrations. This inhibition was caused probably as a result of interference with the action of endogenous auxin. The stimulation caused by IAA at all its stimulatory concentrations was counteracted by TIBA. This is in full agreement with Varga and Humphries (1974). Thus, TIBA establishes itself as auxin-antagonist as suggested by Audus and Thresh (1956).

**IAA-Coumarin interactions**:

Interaction between IAA and coumarin was also studied to explore the possible mutual antagonistic or synergistic effects. The concentration range of IAA was 0.01, 0.05, 0.5 and 1 ppm while that of coumarin was 10, 20, 50 and 100 ppm. The stimulatory effect of IAA was further enhanced by coumarin resulting in synergistic
effects. The disk expansion was promoted significantly by both the compounds commencing from the first growth phase to the end of the experiment. The growth rate increased gradually and reached the peak value during the third growth period. The combined effect of the optimal concentrations of both the compounds (0.5 ppm IAA + 20 ppm coumarin) resulted in producing 34.17, 57.93 and 54.83 % increase over the control after 24, 48 and 72 hr respectively. Thimann and Bonner (1949) reported stimulatory effect of coumarin and a synergistic effect of coumarin and IAA on the growth of oat coleoptile sections. They observed the synergistic effect at low concentration while higher concentration of it inhibited growth. Nitsch and Nitsch (1961) observed synergistic promotion of growth by phenolics only, after adding indole-acetic acid as auxin. Gantzer (1960) recorded a synergistic effect of coumarin and IAA in oat straight growth test. Such synergism between coumarin and IAA has been reported by Samal and Deka (1977b) in bean hypocotyl sections. Thimann and Bonner (1949) and Neumann (1960) envisaged that the site of action of coumarin (or other phenolics) is not identical with that of IAA. Rather, both the compounds compete for inactive sites and cause increased efficiency for auxin. This might result in producing synergistic effects (Thimann and Bonner 1949).

It has been reported that auxin in some way increases the elastic tensility of the cell walls during the first phase of elongation (Purström et al. 1970). It has been suggested that auxin might enzymatically increase the proton concentration in the membranes and thus change the permeability (Uhrström 1974). It was
also supposed that auxin increases ion uptake (Lado et al. 1976) or changes the membrane potential (Tanada 1972). Similarly coumarin may affect the structure of the cell wall or plasmalemma (Svensson 1971, Uhrström and Svensson 1979) and may change the pattern of deposition of cell-wall matters (Albersheim et al. 1977, Burström 1975). Thus, by imparting identical actions on cell walls, IAA and coumarin produced synergistic effects on expansion of bean leaf disks.

**GA$_3$-MH interaction:**

GA$_3$ proved to be a potent stimulator of leaf disks of bean and MH emerged as strong inhibitor. Their possible interactions were explored by using wide range of concentrations of GA$_3$ (10, 20, 50 and 100 ppm) and MH (1, 10, 20 and 50 ppm). All the concentrations of GA$_3$ stimulated growth and 20 ppm emerged as optimal recording 13.85, 16.00 and 18.30% growth stimulation after 24, 48 and 72 hr of incubation. MH inhibited growth and the magnitude of inhibition gradually increased with the increasing concentrations.

During the first growth phase (0-24 hr), GA$_3$ could alleviate the inhibition caused by all the concentrations of MH except 50 ppm. In the next phase (24-48 hr) the magnitude of inhibition at 50 ppm was also further reduced which culminated in growth promotion in the next phase (48-72 hr) at least in one combination (20 ppm GA$_3$ + 50 ppm MH). The overall growth response with GA$_3$ plus MH however was small compared to GA$_3$ acting alone. On statistical analysis, the individual effects as well as their interactions turned out to be highly significant.
The inhibition induced by MH may be due to blocking of some essential reactions such as synthesis of nucleic acids (Baskakov and Putenko 1961) or some other processes that precede the one where \( \text{GA}_3 \) normally exerts its effect (Brian and Hemming 1957, Mathur and Yadav 1975). Addition of exogenous \( \text{GA}_3 \) probably could counteract such actions by meeting the requirement for \( \text{GA}_3 \). Exogenous \( \text{GA}_3 \) might have exerted its effect through its known auxin-sparing mechanism (Arney and Mancinelli 1967, Bednarz et al. 1967) thus countering destruction of IAA by indole-acetic oxidase. Those actions of \( \text{GA}_3 \) on MH resulted in counteracting the inhibition caused by MH. Probably with the rise in concentrations of IAA with the passage of time the magnitude of inhibition declined and even at the highest concentration of MH slight growth promotion ensued with 20 ppm of \( \text{GA}_3 \).

**\( \text{GA}_3 \)-Growth retardant interactions:**

Interactions between \( \text{GA}_3 \) and growth retardants were investigated to have a clearer appraisal of their mutual antagonism. CCC, Phosfon D and B-995 were applied at the same range of concentrations (100, 250 and 500 ppm) and \( \text{GA}_3 \) was applied at 10, 20, 50 and 100 ppm.

The inhibition caused by CCC was alleviated to a great extent by the addition of \( \text{GA}_3 \). After 24 hr certain combinations (100 ppm CCC + 10 ppm \( \text{GA}_3 \), 100 ppm CCC + 20 ppm \( \text{GA}_3 \), 250 ppm CCC + 20 ppm \( \text{GA}_3 \)) the inhibition caused by CCC was completely nullified by \( \text{GA}_3 \) and resulted in growth promotion. In other combinations too, the magnitude of inhibition was relieved to a large extent. The same trend was observed after 48 and 72 hr of incubation. This can also be
interpreted as reduction of stimulatory effect of $GA_3$ thus establishing a clear cut antagonism between the two. Such antagonism between $GA_3$ and CCC was also reported by Cleland and Zeevaart (1970), Marlanegeon (1970), Ross and Pradbeer (1971 a,b), Dunberg and Eliasson (1972) and Sarma and Deka (1977a).

When $GA_3$ and B-995 were combined identical results were obtained. The inhibition induced by B-995 were alleviated to a large extent, but except a few combinations (100 ppm B-995 + 10 ppm $GA_3$, 100 ppm B-995 + 20 ppm $GA_3$) other failed to bring it to stimulatory level. However, a clear cut antagonism was evident between the two compounds. On statistical analysis, the interaction between B-995 and $GA_3$ emerged as highly significant for all the periods under observation. Antagonism between the two was also reported by Williams and Stahly (1970).

Phosfon D acting in conjunction with $GA_3$ exhibited identical results as obtained with B-995 and $GA_3$. But the magnitude of inhibition caused by Phosfon D was so intense that there was only negligible alleviation in combination with $GA_3$. Phosfon D at its lowest concentration (100 ppm) with $GA_3$ exhibited some elimination of inhibition and that too did not reach stimulatory level during all the periods under observation. On statistical analysis, the interaction between the two compounds emerged as highly significant. Such interaction between $GA_3$ and Phosfon D has been reported (Cathey 1964).

Several investigators have reported that treatment of plants with CCC subsequently leads to the reduction in growth which results directly from the inhibition of biosynthesis of GA (Bristow 1966,
Cross et al. 1968, Dale and Felippe 1968, Felippe and Dale 1968, Ryujo and Sachs 1969, Cockerell 1970). Many workers have also found that CCC (or other retardants) block the synthesis of kaurene (Anderson and Moore 1967, Barnes et al. 1969, Robinson & West 1970) and GA biosynthesis (Dennis et al. 1965, Zeevaart & Osborne 1965). Growth retardants are potent inhibitors of enzymes kauren- synthase. This enzyme is responsible for the conversion of trans-geranylgeranylnpyrophosphate (GGPP) to ent-kaurenene. Thus, retardants bring about inhibition of gibberellin biosynthesis (Paleg et al. 1965, Upper & West 1967). The inhibition caused by CCC, Prosion D and B-995 may be attributed to such action on GA biosynthesis.

But, in interaction experiments it has been observed that retardant-treated leaf disks did not respond as expected even to high levels of exogenous  

Growth retardants are also reported to inhibit cell division (Sachs et al. 1960) as well as cell expansion (Sachs and Kofranek 1963). Interactions between growth retardants and the metabolism of auxin and gibberellin have been reported (Ninnemann et al. 1964, Dennis et al. 1965). At least one retardant SADH or B-995 is reported to have inhibited biosynthesis of IAA. The mechanism of action of SADH may be based on the hydrolysis of the compound to unsymmetrical dimethylhydrazine (UDMH), which subsequently inhibits diamine oxidase from converting tryptamine to IAA (Reed et al. 1965). Thus, the
inhibition induced by the growth retardants may be due to inhibition of the biosynthesis of GA or IAA. That the application of GA_3 can alleviate to a great extent the inhibition caused by the retardants was also reported by Nathan and Lockhart (1964), Zeevaert (1967).

**GA_3-Cytokinin interaction (kinetin and BA):**

Gibberellic acid, kinetin and 6-benzyladenine individually proved highly stimulatory on the expansion growth of leaf disks of bean. So, it was decided to examine possible interactions between GA_3 and kinetin as well as between GA_3 and BA.

Kinetin was applied at the concentrations of 10, 20, 50 and 100 ppm while GA_3 was applied at 10, 20, 50 and 100 ppm. Both the compounds individually exhibited highly stimulatory effect. Growth stimulation was recorded as 11.83, 16.52 and 12.92 % and 11.61, 32.10 and 29.12 % at the optimal concentrations of kinetin (20 ppm) and GA_3 (20 ppm) after 24, 46 and 72 hr of incubation. GA_3 in conjunction with kinetin elicited still higher stimulation which was more than additive (synergism). For example, the combination of optimal concentrations (20 ppm kinetin + 20 ppm GA_3) resulted in inducing 34.26, 47.96 and 49.47 % increase over the control after 24, 46 and 72 hr respectively.

BA was applied at the concentrations of 10, 20, 50 and 100 ppm while the range of concentrations for GA_3 was the same as in the interactions with kinetin. BA and GA_3 both imparted highly stimulatory effect. At the optimal concentration of BA (50 ppm), the stimulation was recorded as 23.06, 16.36 & 14.17 % after 24, 46 and 72 hr respectively. Higher stimulation was evident with GA_3 recording.
23.34, 35.61 and 36.71 increase at the optimal concentration (20 ppm). Each combination elicited still higher stimulation resulting in 49.38, 42.56 and 57.10% stimulation at the combination of optimal concentrations (50 ppm BA + 20 ppm GA₃) after 24, 48 and 72 hr of incubation. In most of the combinations the growth was more than additive which confirmed the synergistic effects between the two.

These findings are in conformity with those of Back and Richmond (1971) who observed synergism between GA₃ and cytokinin. They also observed that increased level of one hormone decreased the response of the other. One hormone may affect the endogenous level of the other by modifying its biosynthesis or its rate of degradation (Back and Richmond 1971). Where GA₃ is inactive by itself, in such situations also addition of GA₃ enhances the growth response to IAA and kinetin as was observed in Jerusalem artichoke tissues (Setterfield 1963), in tobacco tissues (Murashige 1966) and in cell suspension cultures of Acer pseudoplatanus (Digby and Waring 1966). Cytokinins and GA₃ appear to have complementary effect (Skoog and Schmitz 1972). In detached leaves application of kinetin and GA₃ is reported to prevent the loss of chlorophyll and rise in ABA (Khar oni and Richmond 1976). Application of kinetin can prevent senescence by retarding the reduction in GA level (Chin and Reevers 1976, Goldschmidt et al. 1972, Reid and Raitten 1974). Thus, cytokinins and GA₃ through their complementary action help each other which is manifested in larger growth stimulation.