DISCUSSION

The present investigation was undertaken to assess the importance of chemical substances viz. carbohydrates, mineral nutrition, auxin, gibberellic acid, cytokinin, inhibitors and growth retardants on the extension growth of the excised root sections of tomato.

An isolated growing segment of a plant part is constantly changing system from the moment of excision to the cessation of growth particularly in its response to any exogenous substance, besides undergoing any structural and biochemical change, during the growing period. This situation becomes far more complicated when they are subjected to unfavourable conditions by introducing exogenous growth substances into the medium. The magnitude of growth and the time relationship of such a system are of fundamental importance and for this reason the experiments were designed to make measurements at regular time intervals.

Organic substances, particularly carbohydrates are thought to be the vital factor in cell extension. They 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 root. Auxins, gibberellins and kinetins are known to stimulate growth of the plant parts particularly in the aerial organs. Another effect on growth and development is exhibited by malic hydrazide, a well known growth inhibitor in a different way. Unsaturated lactones such as coumarin and its analogues inhibit many processes involved in growth and development (2-chlorophenyl trimethyl ammonium chloride (CCC) is known to influence the growth of intact or isolated plant parts in a variety of ways. Therefore it was considered pertinent to investigate the nature of actions of these organic and inorganic compounds on the extension growth of root sections isolated from tomato seedlings.

This study was carried out with two objectives:

(i) to study the effects of individual compounds and nutrient solutions proposed by several workers.

(ii) to explore the possible interactions between the growth promoters and inhibitors/ retardants on the extension growth of tomato root sections.
Carbohydrates

Three carbohydrates, sucrose, glucose and arabinose were applied within a wide range of concentrations effect on root sections. During all the growth periods, sucrose promoted extension growth to a great extent. The magnitude of the growth increased with the passage of time. Glucose also exhibited an identical response and the magnitude of stimulation was almost of the same order as in sucrose. But the stimulation of growth by a pentose sugar, arabinose was less than that of sucrose and glucose. After 96 hr the extension growth of the root sections at all concentrations of arabinose came to a halt. The magnitude of growth stimulation reached peak value at the concentration of 1% for all the three carbohydrates. Above that concentration, the magnitude of stimulation exhibited a sharp declining trend. After 120 hr (i.e., after fourth growth period) from the time of incubation, the percentage of growth at the optimal concentrations of sucrose and glucose at 120 hr after treatment was recorded a 15.7 and 14.0% respectively. This finding illustrates the basic role of carbohydrates in the culture media for the growth of the tissues. This conforms to the view expressed by White (1934) Gaucharet (1955) who established the
essentiality of sugar in culture media.

This finding is in conformity with Gautheret (1945), Street and Lowe (1950), Haiderson (1954) and Thomas, Craigie and Street (1963) who reported sucrose to be superior of all carbohydrates. This was further substantiated by Sarma (1971), and Das and Sarma (1971) who established the superiority of sucrose over other carbohydrates in leaf tissue culture.

Arabinose also appeared to be stimulatory at 24 hr after treatment but with the passage of time the stimulation of growth gradually declined. Street and Lowe (1950), Dormer & Street (1949) reported that arabinose is without nutritive value though not toxic which was substantiated by Sarma (1971).

The penetration of the sugars to the plant tissues may be passive one. The osmotic pressure of the solution of the solution apparently controls this rate of penetration of the solution into plant cell. The three sugars tried, exhibited stimulation in all the concentrations except at higher concentrations. The inhibition at the concentration of 1% may be attributed to plasmolysis caused by higher concentrations which behaved as hypertonic solution. The works of Street and Mc
Gregor (1952) suggested that the superiority of sucrose was because it alone could be absorbed by the root at a rate which did not limit growth and differentiation. Further, the finding that sucrose utilization was associated with the appearance in the medium of both glucose and fructose led to the suggestion that there might operate a surface enzyme or solute carrier system which was sucrose specific and which promoted a phosphorolytic splitting of the glycosidic bond in sucrose and a linked transfer of hexose units to the carrier (Dormer and Street 1949; Street and Lowe 1960). Thomas (1961) also demonstrated that at least during the first 12-24 hr of sucrose utilization by tomato roots there is an equimolar release of its monosaccharide moieties.

This finding is in full agreement with the views expressed by several workers (Butenko 1964, Street 1969, Hanson and Edelman 1972, Hildebrandt et al. 1963, Ross and Thorpe 1973, Ross, Thorpe and Costerton 1973 and others) that supply of carbohydrates even to organs rich in chlorophyllous tissues in vitro is obligatory for growth and differentiation.
Nutrient Solution:

Knop's and Hoagland's nutrient solutions were applied to study their effects on extension growth of excised tomato roots. Stimulation of growth was observed with these two nutrient solutions. But the overall growth stimulation was higher in Knop's solution than that of Hoagland's solution. Nutrient solutions at the strength of IN proved to be the optimal concentration. Both the nutrient solutions were combined with varying concentrations of sucrose. Inhibition was exhibited by 10 N Knop's solution in certain cases. Hoagland's solution at 5 and 10N inhibited growth.

These two nutrient solutions enhanced the stimulation of growth in combination with sucrose. The optimal concentration of sucrose (1%) when combined with the optimal concentration of Knop's solution (IN) resulted in maximum increase of 41.2% over the control. In certain combinations sucrose reduced the inhibitory effect produced by Knop's solution.

The optimal concentration of sucrose in combination with the optimal concentration of Hoagland's solution (IN) produced only 13.6% increase over control.
The stimulation caused by the nutrient solution indicates the basic need of nutrients for the growth of the plant tissue. This is in conformity with the findings of White (1943b) who demonstrated that the macronutrient elements essential for whole plant growth are, as expected, essential for the growth of the excised roots as well. Microelements are essential constituents of enzymes. The studies by Glasstone (1947), Boll and Street (1951), Hannay and Street (1954) and Neales (1959) have revealed that excised roots require all the recognized micro-nutrient elements essential for whole plant growth. Thus, the nutrient solutions in conjunction with sucrose resulted in synergistic effects.

**Indole-3-acetic acid (IAA)**

Indole-3-acetic acid inhibited growth of excised tomato root sections within the range of concentrations (0.01-5ppm) tried. During all the growth periods (24-120 hr) the magnitude of inhibition gradually increased from the lower to higher concentrations. The intensity of inhibition also increased with the passage of time. At the end of the last growth period (120 hr) the inhibition was found to be 5.5 to 15.2% at the concentrations of 0.01 to 5 ppm IAA.
The finding is in conformity with those of Street et al. (1954), Hughes and Street (1960) who reported inhibitory effect of IAA. Hughes and Street (1960) on their studies of the inhibitions of growth of excised roots by IAA showed that IAA when partially inhibitory to growth acts mainly through inhibition of cell extension. These results also suggest that the rate of cell division and the persistence of meristematic activity in the root tips of excised roots was controlled by an auxin which accumulated to a critical supra-optimal level. The recent finding (Chadwick and Burg, 1967) that IAA at a very low concentration (10^{-6} M) causes root tissue to evolve ethylene, coupled with the fact that very low ethylene concentrations inhibit the elongation of roots, makes it at least very probable that the inhibition of root growth by auxins is mediated by the production of ethylene.

**Gibberellic acid**

The present investigation was conducted with GA_{3} within a wide range of concentrations (1-100 ppm). GA_{3} stimulated growth within the range of concentrations tried.
The magnitude of stimulation gradually increased with the rise in concentrations up to 10 ppm (optimum) and then declined resulting in inhibition of growth at 20 - 100 ppm. But with the passage of time the inhibition at 20 ppm and 50 ppm was eliminated. The magnitude of growth at 10 ppm of GA$_3$ was recorded as 4.0, 5.3, 5.6, 7.1 and 7.8% over control after 24, 48, 72, 96, and 120 hours respectively.

That GA$_3$ causes root growth stimulation in plant tissues was reported by several workers (Butcher and Street, 1960; Pecket, 1960; Das Gupta, 1972; Sootschi and Schmehl, 1971).

The stimulation of extension growth by GA$_3$ can be attributed to its manifold activities such as cell division (Sachs et al. 1960, Leivonen, 1958) or cell enlargement (Haber and Luipold, 1960, Haber et al. 1969) or both. The cell-division is associated with rapid cell-enlargement in GA$_3$ treatment plant tissues has been reported (Dure and Jensen 1957, Copper 1958, Feucht and Watson 1958). GA$_3$ is also known to regulate enzyme synthesis and this makes an attractive possibilities for GA stimulation of development processes. Several workers have suggested (Varner and Chandra 1964, Warner et al. 1965, Filner and Varner 1967).
that GA brings about the de novo synthesis of new enzymes and this is associated with the stimulations of RNA synthesis. GA3 is also reported to have increased the level of invertase in stem segments (Kaufman 1965, Kaufman et al. 1968, Varner and Chandra 1964) and amylase in other tissues which results in releasing reducing sugars that might enter and participate in polysaccharide bio-synthesis in elongating or expanding cells. Moreover, the presence of suitable concentrations of sucrose seems to have accelerated the GA3 effect as was reported by Butcher and Street (1960) in root tissues.

Some effects of GA stimulation appear to involve early alternations in the formation of some membrane and membrane components in the cell. Jones (1969) reported an increase in endoplasmic reticulum in barley aleurone sooner than the appearance of a- amylase (within 1 or 2 hour after GA treatment). ..deas (1968, Montague and Ikuma (1975) also envisaged that the increased elongation of Avena stem segments elicited by GA3 is associated with increased formation of cell wall material measured on the basis of dry weight.
Montague and Ikuma (1975) by applying radioactive glucose observed the stimulation of incorporation of 14 C glucose into cell wall synthesis soon after GA$_3$ application. By using synthetic membrane wood and Dale and Raleigh (1972) have demonstrated that the permeability is strikingly increased upon addition of GA. Thus all these effects of GA seem to have synchronised resulting in better growth stimulation.

**Benzyladenine (BA):**

Benzyladenine stimulated the growth of excised tomato root sections relatively at low concentration. In the present investigation BA was applied in a concentrations range of 0.01 to 50 ppm. During all the five growth periods (24 - 120 hr) studied, the stimulation of growth was found only at 0.01 ppm of BA. The rate of growth increased with the passage of time. The magnitude of growth stimulation was found to be 1.6, 3.1, 3.9, 4.2 and 5.3% over control at 24, 48, 72, 96 and 120 hr respectively. A sharp inhibition of growth occurred from 0.1 to 50 ppm of BA. Stimulation of growth by lower concentration of BA has also been
reported by Saashi and Leopold (1969), Usciati et al. (1972), cytokinins accelerate mitosis and cell division (Haber, 1960). Kinetin and BA affect protein synthesis by attaching itself to ribosomes (Berridge et al., 1970). BA is also reported to have retarded the senescence (Fletcher, 1969; Jacoby and Dagan, 1970; Fletcher and Medzhit, 1972) which is associated with the mobilization of metabolites and nutrients (Fletcher et al., 1970). All these actions of BA might have promoted extension growth of root sections.

Maleic hydrazide (MH):

The growth regulator MH was found to inhibit the growth of the plant tissues. In the present investigation MH was applied at concentrations of 1, 10, 20, 50, and 100 ppm. Even at the lowest concentration of 1 ppm of MH, inhibition in the elongation of excised tobacco root sections was observed. This finding substantiates that of Graulich and Haagloop (1954) who observed inhibition in the elongation of younger internode of bean and tomato plants. Butenko and Baskakov (1961) were also of the opinion that $5 \times 10^{-3}$ g/l MH was sufficient for inhibiting the growth of an isolated callus tissues of...
control. The inhibition caused by MH is attributable to its antimitotic behaviour which eventually cause growth retardation (Pilet, 1956). This inhibition by MH is also attributed to the blocking of biosynthesis of nucleic acid in plants. Schoene and Hoffman (1949), Naylor and Davis (1950) reported overall growth inhibition of plants including roots following spraying the plant with maleic hydrazide (MH).

It was further observed that higher concentrations evoked inhibition by interfering with respiration of root tips of various plant species accompanied by lowering the PH of the bathing solution upto 4 (Naylor and Davis, 1951). This inhibition, they believed, might be caused by some kind reaction with the sulfhydryl groups of the dehydrogenases involved in it. This view received support from a few other workers (Muir and Munch, 1953), Isenburg et al, 1951).

**Coumarin**

Coumarin within the range of 10-250 ppm proved highly inhibitory in the present investigation. Even with the low dose (10 ppm) of coumarin, the extension growth of root sections was markedly reduced. The inhibition of growth increased with the rise of concentration of coumarin as well.
as with the passage of time. This finding substantiates those of Audus and Justel (1948), Goodwin and Taves (1950) and Svensson (1972) who reported inhibition of root growth by coumarin. Leopold and Price (1956) envisaged coumarin as a sulfhydryl inhibitor and auxin antagonist. Thimann and Bonner (1949) also held the view that the inhibition of cell elongation caused by coumarin was primarily through blocking the reaction mediated by sulfhydryl containing which function as limiting factor for growth.

The growth inhibitory effect of coumarin might be influenced by some other mechanism apart from inhibiting this enzyme (Audus, 1953). Burstrom (1954) attributed the growth effect on wheat roots by coumarin to be due to altering tensility of the cell wall rendering the walls absolutely rigid. Morgan and Jowell (1970) suggested that the growth inhibition by coumarin might be mediated through the production of ethylene in the treated tissues.

This finding is in sharp contrast with those of Miller and Meyer (1950-51), Thimann and Bonner (1949), Sarma (1971) and Sarma and Deka (1977 a) who reported stimulation of expansion growth of leaf-disks and extension growth of hypocotyl segments of bean by coumarin.
The variation of growth regulation by coumarin is possibly dependent on the concentration and on the species of plants tested (Knypl and Szopa 1958). Again its effects may be different on different portions of the growing machinery (Avers and Goodwin 1956).

CCC or \((2\text{-chloroethyl})_{3}\text{trimethyl ammonium chloride}\).

CCC was applied within a wide range of concentrations \((10^{-5}-10^{-3})\) ppm. Stimulation of growth was observed from \(10^{-5}\) to \(50\) ppm. With the rise of concentrations from \(100-500\) ppm, a marked inhibition of growth ensued. The CCC at the concentration of \(50\) ppm exhibited highest growth stimulation and thus stood as optimal concentration during all the periods under observation. This stimulation of growth supports the findings of Davey et al. (1970) who recently examined the effect of CCC on some physiological processes in \(\text{Phaseolus vulgaris}\) and found that CCC treatment increased the photosynthetic activity of leaves. CCC at suitable concentrations is also reported to have increased the total surface area and the number of leaves in \(\text{Sinapis alba}\) (Humphries 1963). Humphries and French (1965) reported that CCC treatment increased leaf production but reduced total leaf area.
Plaut et al. (1964) and Humphries et al. (1965) reported that root growth of bean and wheat was stimulated if CCC was applied as a foliar spray but was retarded if it was applied to the soil.

Kende et al. (1963) reported that when AMO-1618 or CCC was added to a culture of * Fusarium moniliforme* (known to produce GA) less GA was produced, although growth of the fungus was not affected. The reduced amount of GA in the culture is reported to be the effect of blocking of GA biosynthesis, but not due to appreciable destruction of the GA already synthesized (Minnemann et al. 1964).

Quite contrary to these reports, several workers are reported to have observed high level of GAs after treatment with growth retardants. Van Bragt (1969), Halevy and Shilo (1970), Reid and Crozier (1970, 1972) have shown that CCC treatment can lead to increases in GA levels. This increased GA is probably manifested in growth stimulation.

Interaction between IAA and MH.

IAA and MH were applied together to explore their possible counteracting effect on the extension growth of excised tomato root sections. IAA was applied at the con-
centrations of 0.01, 0.05, 0.5 and 1 ppm while MH was applied at higher concentration ranging from 10 to 50 ppm. Both the compounds inhibited growth and the magnitude of inhibition gradually increased from the lower to the higher concentrations. The combined effects of IAA and MH were manifested in higher inhibition than when they acted independently.

Exogenous auxin inhibits root growth. The classical view, therefore, is that the natural auxins in roots (presumably IAA) occur in supraoptimal inhibitory level. Further addition of exogenous auxin consequently resulted in inhibition of root growth (Cholodny 1931, Lane, 1936, Thimann 1937; Bonner and Koeppli, 1939; Pollock et al 1954). This finding substantiates those of Street et al. (1954) Aberg (1957) and Almestrand (1950) who observed inhibition of root growth by IAA.

Aberg (1953) observed an antagonism between MH and IAA when applied jointly to flax roots. He explained this destruction of auxin in roots by MH as due to accelerating effect upon IAA-oxidase system by this compound. Overall growth inhibition of plants including roots by MH has been reported by Schoene and Hoffman (1949) and Maysor and Davis (1950).

The experimental results of different workers point to the conclusion that the inhibitory effect on growth
of plants by N is not possible due to counteracting effect on the endogenous auxin content (Hillman and Selston 1961). Leopold and Klein (1952) on the other hand viewed N to be a direct inhibitor of the action of auxins in promoting the extension growth 'Anti-auxin' activity of N was also reported by Sarma and Borah (1973) on the extension growth of bean hypocotyl segments.

**Interaction between GA₃ and CCC**

Gibberellic acid and CCC were co-applied to examine their possible interactions on the extension growth of excised tomato root sections. Stimulatory range of concentrations of GA₃ (1-50 ppm) and stimulatory as well as inhibitory range of CCC (10-250 ppm) were applied GA₃ at 10 ppm and CCC at 50 ppm. Study of optimal concentrations during all the periods under observation. The combined effect of GA₃ and CCC was found to be higher at stimulatory concentrations of both than their individual effects. The highest stimulation of growth rate was observed when the optimal concentrations of GA₃ and CCC were combined. Thus, the stimulation was recorded as 2.6, 14.3, 19.2, 26.1 and 30.8% over control at 24, 48, 72, 96 and 120 hours respectively. From this it was observed that with the progress of time the growth rate was also increased. That GA₃ stimulates root growth has also been reported by Richardson (1957) and Palsg (1965). This finding also substantiates those of Decker (1960), Buscher and Street (1960) who observed stimulation of excised pea and tomato roots by very low concentration of GA. This stimulation may be attributed to the cell division or elongation or both. That GA₃ at a low concentration did not inhibit mitosis was re-
Detection of GA in cultured tomato roots (Butcher, 1963), root exudates of sunflower (Phillips and Jones, 1964) and grape vines (Skene, 1967) strongly supports the idea that GA might be involved in the growth of roots. On the other hand, the stimulation of growth by CCC may be attributed to increased GA, in CCC-treated plant tissues (Van Bragt, 1969, Halevy and Shilo, 1970, Reid and Crozier, 1970-1972). The combined effect might have resulted in synergistic effects.

Interaction of GA and MH

GA$_3$ and MH were co-applied to explore their possible counteracting effect on the extension growth of excised tomato root sections. GA$_3$ was applied at the concentrations of 1, 10, 20 and 50 ppm, and MH was applied in the same range i.e. from 1 - 50 ppm. GA$_3$ stimulated growth at 1 and 10 ppm. MH within its range inhibited growth and the magnitude of inhibition gradually increased from the lower to the higher concentration. The inhibition caused by MH was to some extent eliminated by GA$_3$ and thus in certain concentrations the combined effect of GA$_3$ and MH resulted in growth stimulation. However, the overall stimulation was lower than GA$_3$ effect counted alone.

Kato (1958) applied gibberellin A in combination with MH to the cucumber seedlings. Shoot growth inhibition of cucumber was found to be alleviated by GA, while the root growth inhibition could not be reversed.

Brian and Hemming (1957 a) reported MH to have
inhibitory effect on pea stem extension opposite to those of GA, thus preventing GA- response to GA sensitive plants. They suggested that MH inhibits growth by blocking some essential reaction at a stage preceding that where GA normally exerts its effect. Haber and White (1960) also found absence of competitive interaction between GA and MH on lettuce seed germination and wheat seedling growth. MH seems to exert direct influence in cell division and not cell expansion during the seedling growth. Therefore, it is apparent MH effects mitosis in a system where GA has no influence and GA is active in cell expansion on which system MH has no effect. G· and MH in combination, they observed, act independently on growth and not through a common mechanism atleast in the extension growth of root tissues.