DISCUSSION

In the air dry seed the water content is so low that metabolic activity is almost at a stand still. When imbibition is initiated at higher temperatures (27°C-30°C) the moisture level of the seed rapidly increases during the first 24 hours. Imbibition of water by the embryo and the endosperm continues even after 48 hours but at a slower rate. There is sufficient evidence to suggest that in the initial stages of germination the elongation of the embryonal axis is mainly brought about by the imbibition of water and not by any cell division or addition of dry matter. The intake of water by the seed brings about changes in the metabolic activity of the embryonal axis. Respiratory activity is accelerated and continues to rise with the advance of germination. This is also governed by the temperature of germination.

Presumably there should always be a time lag between the initiation of imbibition and mobilization of nutrients from the endosperm by the activation of the enzymatic processes. The results leave no doubt for the fact that the major centre of enzyme activity is the embryo and not the endosperm. As the embryonal axis was separated from the cotyledons without moistening the seed there could not have been any transfer of enzymes from the latter to the former. Respiratory process as well as fresh
weight increase during early germination are exponential. This suggests that the developmental process during germination is autonomous (self-regulatory). With an increase in water content of the seed the radicle begins to elongate and at the same time metabolic activity is also accelerated. This is an essential condition for continuing active growth.

During this period of development reserves are mobilized and synthesis of necessary cell constituents takes place, ultimately leading to rapid growth. As seen from the results given in Figs. 31, 32, rate of respiration rises with the imbibition of water and advance of germinative processes. The fact that appreciable level of respiratory activity is reached even after three hours of imbibition and the clear cut difference in fresh weight obtained under the influence of ascorbic acid as well as other growth regulatory substances only 7 hours after the commencement of the experiment suggests that there is an 'active' absorption of water during germination. Earlier reports indicate that ascorbic acid in some way influences imbibition of water by roots. In the present experiment (Table 11), it is quite clear that ascorbic acid metabolism has a profound influence upon the absorption of water. The increase in fresh weight as well as elongation of embryonal axes are both exponential. The respiratory activity also appears to be exponential. James and
James (1940) as well as James (1953) have shown in the case of barley germinated at 20°C that CO₂ production increases exponentially over a period of 48 hours of germination. Spragg and Yemm (1959) obtained similar results in the case of germinating pea seeds. These investigators were able to further demonstrate that in the first stages of germination the seed coat limits the access of O₂ to the embryo and therefore a considerable part of the respiratory CO₂ evolved during early stages of germination may be derived from anaerobic metabolic processes which is most likely alcoholic fermentation.

The position as regards the germination of excised embryos, however, seems to be quite different. But as shown in Table 10, the growth of excised embryonal axis is quite rapid. The early production of ascorbic acid as well as its early utilization in the embryonal axis lends support to our conclusion that ascorbic acid metabolism plays an important role in the initial germinative processes even during the first few hours after the start of imbibition of water. Further it has already been shown in the case of wheat, which has not got the same silicious coat as the barley, that with the imbibition of water ascorbic acid production and utilization starts in the embryo during early hours of germination (Chinoy and Nanda, 1959).

These workers were also the first to show that auxin (IAA) catalyzes the biosynthesis of ascorbic acid in germinating wheat embryos. Experimental data presented by Michniewicz
and his coworkers (1966) also support the above mentioned finding. Recently Chinoy et al. (1965) have also shown that short duration incubation of root tips as well as coleoptile tips in substrates containing IAA and GA with sucrose enhances the biosynthesis of ascorbic acid as well as accelerates its utilization in the growth processes of the seedling. The present findings also corroborate the above mentioned conclusion, viz., that growth regulators like IAA, GA and others have an enhancing effect not only upon the biosynthesis of ascorbic acid but also upon its utilization (Fig. 5, 10). The present writer is therefore inclined to the view that the effect of these growth regulators on processes of growth during germination is an indirect one, viz., by their influence on its ascorbic acid metabolism. Mapson and Moustaffa (1956) have shown that full ascorbic acid oxidase activity is attained only after three days of germination in pea seed. Spragg and Yemm (1959) believe that although ascorbic acid as well as glutathione are present in germinating pea seeds the ascorbic acid - glutathione oxidation reduction system does not play a significant role in respiration during early stages of pea germination. In the case of excised embryo, however, due to the free and easy access of oxygen ascorbic acid metabolism gets fully activated from almost the beginning of the imbibition of water and appears to fully participate in respiration as an electron donor in the
oxidative phosphorylation as well as in the resulting growth as seen from the synchronization of ascorbic acid concentration and its utilization with the evolution of CO₂ and elongation of the embryonal axes (Table 10, 12 and 14).

It becomes clear that the same succession of phases of development takes place in an excised embryo as in an intact seed. In the case of the former, however, the first phase is very much shortened on account of the removal of the impediment of seed coat to the entry of water and air into the embryo. It is also clear that the succession of these phases is not in any way governed by the supply of nutrients from the endosperm because it takes place in an excised embryo also. The process appears to be an autonomous one arising from the development of the embryo itself.

Raadts and Soding (1947) and Raadts (1948) were able to demonstrate that ascorbic acid was able to produce curvature in Avena coleoptile in day light. It promoted the rate of growth without influencing the final length of the coleoptile. Ascorbic acid also enhanced the activity of the auxin. Raadts (1948) has put forward the suggestion that ascorbic acid accelerated the conversion of inactive growth hormone into the active form. Künnen and Soding (1949) and Künnen (1950) were able to induce cambial activity in the decapitated stumps of Helianthus
epicotyl within 2-4 weeks by applying 0.05 mg. ascorbic acid in lanolin. Thiamin (0.04 mg.) was also equally effective. These workers ascribe cambial forming property to ascorbic acid and thiamin.

Newcomb (1951) has reported a considerable enhancement in ascorbic acid oxidase activity of both root and shoot tissues just before the initiation of cell enlargement caused by auxin. As the enzymatic activity was mainly localized in the cell wall fraction of tissue preparations this investigator suggested that AAO played some specific role in the active growth of the primary cell wall.

Honda (1955) also found active ascorbic acid oxidase in the cell wall fraction of tissue homogenates prepared from the roots of 5-day-old barley seedlings. Earlier Reid (1941a) found a direct correlation between ascorbic acid content and cell size in the developing root of cowpea seedling. She was able to establish a correlation between cell surface area and ascorbic acid concentration at different stages of development.

Growth regulators during vernalization period: Purvis and Gregory (1937) were able to show a quantitative effect of vernalization treatment in winter rye. These workers determined the optimum duration of thermoinduction. Lang (1951) demonstrated in the case of *Hyoscyamus*
that the optimum in thermoinductive process shifted with the range of temperature used for the vernalization treatment. The above fact lends support to the findings of Razumov (1961) that thermoinduction initiated at a relatively high temperature can continue even at as low a temperature as -6°C, which by itself is not thermoinductive. Nanda, Chinoy and Sirohi (1958, 1958a, 1958b) have also worked out a quantitative relationship between the duration of vernalization as well as photoperiod.

The quantitative nature of vernalization can also be confirmed by the sensitivity of the thermoinduction process to high temperature. Devernalization effect of high temperature has been observed by a number of workers in different types of plants, such as sugarbeets (Stout, 1945; Curth, 1960), Petkus winter Rye (Purvis and Gregory, 1952), Hyoscyamus (Lang and Melchers, 1947), Arabidopsis (Zenker, 1955; Napp-Zinn, 1957), radish (Tashima, 1957); Chrysanthemum (Schwabe, 1955), peas (Highkin, 1956) and others.

Besides high temperature other conditions are also required to bring about devernalization. Thus for instance Purvis and Gregory (1952) showed that devernalization of Petkus winter rye could only be brought about under restricted watering which actually prevented germination. Seedling growth in some way prevented devernalization. Further a number of workers have observed
progressive decrease in the reversion of cold temperature effect by prolonging the period of vernalization (Avakyan and Yastrebov, 1949, Kurth, 1954; Razumov, 1961).

There is evidence to show that during thermoinduction some transmissible substance is produced which can pass through a graft junction. Welchers (1939) has given the name of vernalin to such a substance and has considered it as an essential step for the formation of florigen.

Considering the mechanism of vernalization it can be said that carbohydrates are the first essential prerequisites for vernalization. This fact is brought out clearly in experiments with excised embryos of winter cereals (Kurth, 1938; in Petkus rye; Konovolov, 1937, in wheat; Tashima, 1957 in ecotylized embryos of radish).

Purvis (1944, 1947, 1948) has studied in detail the effect of various sugars on vernalization of winter rye and came to the conclusion that sugar is needed in the synthesis of a material which is necessary for flower induction. In entire grains this is supplied by the endosperm. This substance is very susceptible to the influence of high temperature. It has however been pointed out by Purvis (1947, 1948) that even if the excised embryos are cultured in sugar-free medium for some days prior to cold temperature treatment, high level of vernalization is brought about in rye, thus suggesting that in rye embryo there is sufficient amount of substances needed for the vernaliza-
tion process, which, of course, proceeds at a reduced rate. Oxygen is also essential for the successful completion of vernalization (Gregory and Purvis, 1938a, 1938b; Günther, 1959-60, Filippenko, 1940, Tashima, 1957).

It is claimed by a number of workers that gibberellin induces flower formation in cold requiring plants under long-day conditions (Lang, 1957; Carr et al. 1957; Chila-khyan 1957). Under short day conditions elongation of the shoot may take place but flowering does not take place. There is, however, contradictory evidence on this point. In many cases gibberellin treatment failed to initiate flowering as well as stem elongation. In some cases response to gibberellin was obtained only after the application of a massive dose of the growth regulator (Lang, 1957; Chouard, 1957, 1958). In some cases gibberellin became effective only at a temperature which was very close to the thermoinductive one (Bukovac and Wittwer, 1957; Gaskill, 1957 in beets; James and Lund, 1960; Razumov et al. 1960 in winter cereals like wheat, barley, oats).

Lang (1965) has summarized the responses of many plant species to gibberellin obtained by various workers and has concluded that seed treatment with gibberellic was ineffective in the induction of flowering even in seeds which were highly responsive to vernalization treatment. Further he has cited the work of Tsukamoto and Konishi (1959) on radish and Razumov et al. (1960) on winter wheat.
to show that presence of gibberellin during thermoinduction reduces the effectiveness of the latter.

Although a number of workers like Harada (1962), Chailakhyan and Lozhnikova (1962), Chailakhyan (1961, 1964) and Michniowicz (1966) have reported an increase in the endogenous gibberellin-like substances in plants after thermoinduction, one is inclined to agree with Leng (1965) that the inability of gibberellin treatment during vernalization as well as in fully thermoinduced plants suggests that gibberellin does not play any direct role in thermoinduction. He, however, sees a possibility of gibberellin functioning in cereals mainly after the initial stages of flower initiation.

**Auxin Action:** Cholodny (1936) was the first to postulate that during vernalization the embryo absorbed considerable quantities of growth hormones from the endosperm which, on account of restrictions imposed upon growth during vernalization, accelerated the developmental process of the cells in the apical meristem, thus leading the plant to early flowering. A little later he (Cholodny, 1939) put forward the hypothesis that the initiation of organs of sexual reproduction is determined by the action of a certain complex of phytohormones (auxins, vitamin B and others) and not by the action of a single specific flower forming substance. Michniewicz et al. (1966) as well as Michniewicz and Kamionska (1966) have discussed the
question of differences in auxin level between spring and winter varieties. Free and bound auxin content was tested in five spring and winter wheat varieties, considerable changes in auxin level were observed during the process of germination. These changes were, however, found independent of the spring or winter habit of the variety. No difference was also found in the concentration of growth promoting substances of vernalized and non-vernalized kernels. These results led these investigators to conclude that vernalization was not dependent upon changes in the auxin level during the chilling of kernels. They have further suggested that growth inhibitors may play a significant role in the process of vernalization.

Stanislawski (1966a) studied the 3-indolylacetic acid content of vernalized and non-vernalized winter as well as spring wheat varieties with special reference to the effect of devernalization. During the first 20 days of germination there was no significant difference in the dynamics of 3-indolylacetic acid in all the varieties and treatments. Raising the temperature of germination increased the concentration of 3-indolylacetic acid both in vernalized as well as non-vernalized winter and spring wheat.

As already pointed out earlier in the thesis Lang (1960) considers gibberellin as a physiological precursor, and not a biochemical one of florigen. Further
Chailakhyan (1961) has modified his original florigen concept by assigning interacting roles to gibberellin and anthesines. He considers that gibberellins plus anthesines produce florigen. In the light of all the evidence cited above it is very difficult to comprehend how this can be so. Although there are many claims regarding the presence of gibberellin like substances in higher plants determined on the basis of various bioassays, very few attempts have been made for obtaining confirmatory biochemical evidence for the various types of gibberellins now known. Unless that is done in the case of a large number of plant species it would be premature to postulate about the universal presence of gibberellins in higher plants; and therefore at best this question of the actual universal presence of gibberellins in plants must remain mainly a hypothetical one. Further the presence of vernalin as well as anthesines also remain mainly a hypothetical one. Further the presence of vernalin as well as anthesines also remain more nebulous than that of gibberellin.

Chailakhyan and his co-workers (1962) were able to grow mature plants by culturing growing shoot apices on a medium containing nucleotides and nitrogen bases. On the basis of this evidence Chailakhyan (1964) has postulated that anthesines are nitrogenous compounds of nucleotide composition. Although it is not clear from his work whether these nitrogenous compounds (anthesines) needed
for flowering were similar to the nucleotides required
during the growth of vegetative organs.

Vernalin concept of Melchers (1939) and Melchers
and Lang (1941) is equally hypothetical. Further if we
consider the confused state of information regarding the
physiological role of auxins (Galston and Pervez, 1960;
Thimann 1963 and others) and similarly that of gibberellins
(Lang, 1965, 1966; Stowe and Yamaki, 1957 and others),
it becomes abundantly clear that it is very difficult to
assign a clear cut direct metabolic role to auxins and
gibberellins in such fundamental process of the plant as
growth and flowering. Even in those cases where the
presence of these growth regulators in plants has been
proved conclusively by biochemical tests they have always
been found in sub-micro concentrations suggesting a
catalytic role for them in plant processes.

Evidence from grafting experiments, where a flowering
stimulus is shown to pass from an induced leaf to a plant
kept in noninductive temperature and photoperiod is so far
the only experimental basis to infer the presence of
florigen. A number of attempts have been made to extract
plant material for obtaining 'florigen' in a pure state
(Lincoln, Mayfield and Cunningham, 1961; Lincoln et al.
1962; Mayfield et al. 1963; Lincoln, - 1964). These
workers succeeded in obtaining crude extract from flowering
Xanthium pensylvanicum which when applied to non induced plants of the same species caused initiation of flowering in some of them. The effects were however not very conclusive. Generally only 30% of treated plants showed indications of flowering and some of the experiments gave negative results. Further no control extracts from non-induced plants were tried to see whether they gave negative response. In later work Lincoln (1964) has tried to fractionate extracts from various plants and succeeded in obtaining an acidic fraction which they have named as florigenic acid. This acid although not in a completely pure form, gave a positive response in non-induced plants.

In this connection it would be worthwhile to mention the work of Bonner et al. (1963) on the suppression of photoinduction in Xanthium and Pharbitis by the application of tris-(2-diethylaminomethyl) phosphate trihydrochloride and also its dimethylaminomethyl analogue to leaves without reduction of growth or any other injury to the plant. On the basis of these experiments these investigators have assumed that the above mentioned substance interfered with the synthesis of florigen. As this chemical is known to be a powerful inhibitor of the synthesis of sterols in animals an interesting possibility of florigen being a sterol is put forward by those workers.
Lincoln and his coworkers to extract and identify florigen the question of providing conclusive evidence for its existence in plants remain unanswered.

**AA and vernalization:** Evidence is accumulating to show that during germination of seeds, inductive temperature (30-5°C) brings about an enhancement in the ascorbic acid content. Shrinivasan and Wandrekar (1950) were able to demonstrate the beneficial influence of steeping the seed in the cold on its AA content. Germination in the dark also enhanced the AA content. Bhurani, Shah and Shrinivasan (1953, 1953a) have also carried out comprehensive experiments on the biosynthesis of vitamin C during germination. These workers have also demonstrated the beneficial effect of cold treatment of seeds on their AA content.

Chinoy, Nanda and Garg (1957) have shown that AA treatment of the growing points of plants for a few hours during the early stages of its growth results in enhancement of growth and acceleration in flowering at a much later period in the life of the plant. From the above they have concluded that the effect of AA treatment is an inductive one "analogous to that of vernalization". Chinoy, Singh and Sirohi (1957) showed that vernalization treatment combined with long photoperiod brought about an earlier upsurge in the level of AA in the shoot apex of Hansa (Triticum aestivum) a winter variety obtained
from Svalof, Sweden, through the kind courtesy of Professor Arne Muntzing, Director, Institute of Genetics, University of Lund, Sweden. A significant increase in AA concentration synchronized with the change in the shoot apex from vegetative to reproductive state. A low level of auxin corresponded with this high level of AA.

Chinoy and Nanda (1959) reported an early and rapid rise in the concentration of AA in a medium containing both IAA and sucrose. This increase due to IAA plus sucrose was especially well marked in sets germinated at low temperature. These investigators considered this enhanced biosynthesis of AA by wheat embryos at low temperature to be significant from the point of view of mechanism of vernalization.

Zakharshichina (1960) has shown in the case of multiple grats of tomatoes and egg plants that the transition of these plants from the vegetative growth phase to the reproductive development was accompanied by accumulation of ascorbic acid and nucleic acids in leaves. With the approach of flowering the DNA/RNA ratio was found to shift towards DNA.

Michniewicz (1961) determined AA content of grains of three varieties of winter wheat at different stages of vernalization. AA content of leaves of vernalized as well as unvernalized plants was determined according to their
age, growth and development stage. He found that AA content of vernalized grain was higher compared to unvernalized seed at the same growth stage. Plants grown from vernalized seeds registered higher AA content than control plants of the same age. Ascorbic acid continued to increase during the course of the ontogenetic development reaching its maximum just before blossoming and thereafter declined.

Michniewicz and Rowicka (1961) found that ascorbic acid content increased with the progress of germination. Differences in AA content depended mainly upon the physiological condition as well as on the stage of growth and development of the plant. It was, however, not affected by the spring or winter habit of the variety.

In the light of data presented in the thesis as well as taking into consideration what has been said above regarding the role of auxins and gibberellins in vernalization the present writer is inclined to the view that the action of these 'hormones' is an indirect one mainly that of catalysts. Indole-3-acetic acid and gibberellin accelerate not only the production of ascorbic acid but also its utilization in excised embryos (Figs. 5,9,10). This is a significant fact which has also been previously observed by a number of workers not only in seeds and excised embryos, but also in isolated sections of different plant material like coleoptile and root tips. (Chinny and
In this connection it would be of interest to re-examine the reported action of auxin in presence of sucrose as well as in its absence. As early as 1938 Thimann and Schneider (1938) and Schneider (1938) showed that maximum growth in *Avena* coleoptile sections could be obtained in auxin solutions containing one per cent sucrose and 0.01 M KCl. On the basis of these observations they recommended the use of both these substances in the medium for the *Avena* coleoptile section straight-growth bioassay of auxin. On the other hand Bentley (1950, 1958) obtained very variable results with the use of sucrose in the medium and therefore did not recommend its use as a food factor in the straight growth test. This uncertainty about the role of sucrose arose due to the lack of proper understanding about its role and also about the manner in which auxin participated in the growth reaction. Chinoy, Grover and Sirohi (1957) have shown that growth of *Avena* coleoptile sections practically stopped after 12 hours in sucrose-free auxin medium. In auxin solutions containing sucrose, on the other hand, length of coleoptile sections increased right up to the end of the experiment. Another point of interest was that the *Avena* coleoptile sections placed in one per cent sucrose solution invariably surpassed in growth those placed in $1.79 \times 10^{-7}$ molar concentration of IAA. This led
(Ghinoy, et al. 1957, 1961, 1965) to determine whether sucrose of the test medium was serving merely as a food factor or whether it also served as a precursor of a growth regulator. When different concentrations of sucrose (0.1 and 1.0 percent) were used, appreciable enhancement in linear growth of Avena sections over the non-sucrose series took place only in media containing 1.0 percent sucrose. The enhancement in growth was not so marked in the case of low concentration of sucrose (0.1 percent). Even at low concentration sucrose should have served adequately as a food factor. Part of the enhancement in growth in a medium containing 1.0% sucrose was therefore considered to be due to the biosynthesis of a growth-regulating substance from sucrose. This postulate was confirmed by studying the growth-auxin relationship by the method of enzyme kinetics as well as by actual determination of ascorbic acid content in embryos and explants of plant organs (Chinoy, et al., 1965). In the present work also considerable evidence is presented to show that the role of auxin and gibberellin is to stimulate not only the synthesis of ascorbic acid but also to activate enzyme systems utilising ascorbic acid as a substrate. (Fig. 10).

During germination process soluble carbohydrates, amino acids, fatty acids, nucleotides etc. are formed and translocated to the embryo in preparation of growth. As
the mobilisation of carbohydrates increased, the synthesis of ascorbic acid takes place. Further free auxins are also released from the bound state and they in turn catalyze the biosynthesis of ascorbic acid (Chinoy, 1959). There are sufficient evidences to support the fact that AA concentration rises earlier in the embryo subjected to vernalization treatment. (Chinoy, and Nanda, 1959; Michnie-wicz, 1961). It is also found from the data presented in the thesis that along with ascorbic acid content, ascorbigen content is also high during early stages of germination (Figs. 33-36, 39-40, 43-46, 49-52). It decreases with the march of germination which indicates that ascorbic acid is utilized during germinative process. From the data it is also found that more ascorbic acid is utilized in the embryo than that in endosperm (Figs. 37, 38, 41, 42, 47, 48, 53, 54), which again shows that embryo is the major seat of enzyme activity.

A mass of data on different crop plants reveals the important role of ascorbic acid in growth and development. Vernalization and long photoperiod help in raising the level of AA at an earlier date in the shoot apices as compared to the level of AA in the shoot apex of corresponding NDC plants.

Ascorbic acid production rises together with its utilization; but as the rate of production is faster compared to the rate of utilization its concentration
steadily increases, thus maintaining the redox balance towards the reductive side which is necessary for maintaining a high rate of synthesis of cell constituents required for growth.

Under long day conditions and vernalization this upsurge in the AA content as well as its utilization is brought about much earlier compared to NDC and NDV treatments. (Figs. 55, 57, 61, 63). Due to the enhanced metabolic activity the primordia are laid down in rapid succession on the shoot apex thus bringing about transformation of shoot apex from vegetative to reproductive differentiation. Throughout the period of reproductive differentiation the redox balance is maintained well on the reductive side by the high level of ascorbic acid in spite of its rapid utilization. It is necessary to take into account the ascorbigen content which shows high level during vegetative period of differentiation and low level during reproductive period of differentiation (Figs. 56, 62). It indicates that with rapid differentiation of the various organs of the spike in preparation of flowering, ascorbic acid which was accumulating in a bound state during the period of vegetative differentiation is released in a free condition in the shoot-apex for use in active metabolism.

Further, in the case of leaf also high ascorbic acid and ascorbigen content are found in the initial stages of
vegetative differentiation. At the transformation stage ascorbic acid as well as ascorbigen content decreases whereas utilization is at a high level (Figs. 58, 59, 64, 65). Thus ascorbic acid is synthesized in the leaf during vegetative period and part of it is stored as ascorbigen and then translocated in large quantity to the apex for use during differentiation of the reproductive organs. The higher rate of the enzyme activity in the leaf (Figs. 60, 66) during reproductive differentiation signifies the enhanced synthesis of building material for proteins and other macro-molecules, essential for the increased production of growth centres during rapid differentiation of different organs of the spike and florets.

It has been shown earlier that the formation and utilization of ascorbic acid stimulates the production of nucleic acid, which in turn increases the production of proteins and protoplasm and as a result organizer centres are laid down and cell division and cell enlargement take place. This brings about the change in the norm of mitotic division and gives rise to meiosis (Chinchy, 1962).