In the present study an attempt has been made to elucidate the effects of heavy water ($D_2O$) on physiological processes in higher plants represented by barley (Hordeum vulgare c.v. 292). Various experiments undertaken in this connection and compiled in the form of this thesis are interrelated and complementary in most cases.

Growth, a macroevent in any organism, is a terminal manifestation of many microevents. The microevents include cell division, cell elongation, transport and synthesis of metabolites, etc. Heavy water has been used as a tool of great value in studying physiological phenomena in most of the organisms through study of changes in enzyme reaction rates. Interference by heavy water in any of the physiological processes, should, therefore, influence the morphology of the living beings. Absorption of water is the first process which initiates metabolic activity during seed germination. Onset of enzymatic activity, acceleration of respiration, changes in DNA, RNA and protein contents are some of the earliest sequential reactions marking initiation of germination. Application of $D_2O$ is seen to adversely influence the physiology of seed starting from the hydration phenomenon itself. Poor hydration due to $D_2O$ can slow down almost all the above said processes, thus causing a delay in germination process of seeds.
Growth in barley seedlings has been expressed in terms of fresh and dry weights accumulation and elongation of shoot and root. Effect of heavy water has been seen to inhibit drastically all these growth characteristics depending upon the concentrations. From these studies it may be concluded that the isotope deuterium, through its multifarious fields to effect, finally brings about changes in morphology of a plant. Changes in dry weight of different organs are reflections of altered metabolic status due to D₂O. However, greater inhibition in shoot and root elongation compared to the corresponding dry weight accumulation in plant organs has been assumed to be due to D₂O-induced retardation in cell division or elongation more than the break-down and/or transport of reserve materials from endosperm to the growing axis. Histological examination of barley seedlings during a test period of 7 days showed a D₂O-caused delay in the appearance and subsequent development of different leaves. D₂O seems to have arrested meristematic activities corresponding to longitudinal, radial and transverse expansion of leaf. Based on physiological studies detailed in subsequent Sections these effects are attributed to the retarded metabolic processes of the plant in the presence of heavy water.

Morphological growth of a plant is the final expression of any change in the regulation of metabolism. If life has to
process in an orderly fashion the flow of metabolites through diverse biosynthetic and catabolic pathways must be regulated within the cells. Examples are energy production, interconversion of metabolites and production of precursors for resynthesis, etc.

Replacement of hydrogen by deuterium represents an environmental alteration that the tissue might have to contend with, if it is to survive. In such a condition all the above mentioned processes must coordinate and in addition must suitably respond to changes in external environment. Therefore, the physiological studies related to carbohydrate, protein and energy metabolisms have been taken up to estimate D$_2$O effect on all these processes, finally reflecting on morphological growth.

Preliminary studies on total nitrogen and sugars in endosperm and embryo of barley seedlings during a period of 4 days clearly showed an imbalance in protein and carbohydrate metabolisms due to heavy water treatment. Amino acid data reveal a block in the breakdown of the reserve proteins in the seeds and/or interference in the movement of solubilized material to growing regions. Proteins, forming about 10% of the dry matter, constitute one of the important reserve materials of barley seed. Various types of proteins from these seeds have been extracted with different solvent systems. During germination and growth, heavy water is seen to retard the changes taking place in all types of proteins and more significantly in the salt soluble ones representing the enzyme proteins especially the de novo synthesised ones. This fact is well confirmed from
measurement of protease and $\alpha$-amylase both of which are de novo synthesised in the aleurone cells of barley seeds endosperm. Moreover, their synthesis is dependent on gibberellic acid (GA$_3$). The latter is known to be produced in embryo of barley seeds. On hydration of seeds it gets diffused into the aleurone layer where the mRNA is formed specific for synthesis of these enzymes. Our own results on protease and $\alpha$-amylase activities with whole seedlings have shown that D$_2$O exerts its effect at almost all steps of enzyme synthesis. Subsequent studies are done with water and heavy water soaked half seeds (embryoless endosperm halves) and with various concentrations of externally applied GA. These experimental results indicate that, up to 50% D$_2$O level, it is only the rate of reaction of this enzyme which is affected but at 100% level, heavy water is seen to suppress the synthesis of GA itself. That the $\beta$-amylase activity has not been significantly retarded confirms the fact that heavy water does not hinder the release of the bound $\beta$-amylase. But it definitely proves that de novo synthesis of certain enzymes will either be slowed down or completely arrested, at least for a longer period, after soaking. Starch is known to be one of the major constituents of seed reserves of barley forming about 65% of the dry weight. Moreover, $\alpha$-amylase is said to account for nine tenth of the amylolytic activity while $\beta$-amylase would carry out the remaining one tenth. From the above experimental evidences it is noted that D$_2$O interferes in the de novo synthesis of enzymes drastically and therefore, major portion of starch can be expected to remain
unhydrolysed. Referring to changes in these two constituents namely proteins and carbohydrate, D$_2$O effect can be speculated mostly on metabolically active proteins (i.e. salt soluble) which include the enzyme proteins. If latter's formation is arrested, hydrolysis of metabolically inactive (reserve) proteins and starch hydrolysis are likely to be hindered.

In all these studies, the seed germination and subsequent seedling growth have been obtained in an etiolated condition without any external source of nutrient. In such cases, it is proper to say that the embryo grows only at the expense of endosperm. Hydrolysis of endosperm reserves and the transport of the same to the embryo are the two important determinants for seedling growth. From present studies, it is ascertained that heavy water hinders both these processes. This fact is evidenced from changes in dry weight of endosperm and embryo. Also, that the D$_2$O inhibits the transport more than the hydrolysis of metabolites has confirmatory evidence from our physiological studies.

The studies, included under energy metabolism experiments, are undertaken with a view to correlate the observed decrease in growth rate, when plants are raised in 50% D$_2$O, with a probable decrease in the energy metabolism of the mitochondria. These studies covering oxidative phosphorylation, ATPase activity and substrate respiration do not show any specific alteration in the mitochondrial functions. Only enzyme reaction rates seem to be slowed-down due to replacement of H by D. In general, not much
correlation appears to exist between $D_2O$ effect on energy metabolism and growth rate. It will, therefore, seem that stunted growth in 50% $D_2O$ does not result because of impairment in the energy metabolism of the mitochondria as such and hence several other possibilities present themselves. These include 1) net ATP formation, 2) utilization of ATP in energy dependent growth processes and 3) level of other growth promoting substances. It is also likely that the content of mitochondria may be less in the $D_2O$ grown plants, thereby resulting in a net lowered synthesis of ATP. Further, the enzyme systems which utilize ATP for promoting growth may be affected severely when plants are grown in heavy water resulting into slow growth rates. With reference to the level of growth regulators, there is a possibility that $D_2O$ treatment may interfere with the levels of GA. In fact, our studies (GA dependent enzyme synthesis, experiments with half seeds) have shown that the (inhibited) enzyme synthesis in $D_2O$ grown plants can be promoted with higher concentrations of GA. An in-depth investigation of these possibilities will be of help in understanding further the observed effects of $D_2O$ on plant growth.

In brief, from the studies undertaken during this period of experimentation, $D_2O$ is found to significantly affect cell division and elongation causing delayed germination and retarded growth at early hours of morphological measurement. Physiologically, $D_2O$ seems to affect growth at relatively later stages, resulting into reduced rates of hydrolysis and transport of solubilized material. Retarded protein and carbohydrate metabolisms are found to be rather major causes for stunted growth of barley seedlings as compared to the change in energy metabolism.