Section I: GENERAL INTRODUCTION AND REVIEW OF LITERATURE
Deuterium, a stable isotope of hydrogen was discovered by Urey and his co-workers in 1932. The greatest mass ratio existing between any two stable isotopes is between hydrogen (protium) and deuterium, the latter being double in mass than the former due to the presence of an additional neutron. The corresponding compounds of hydrogen and deuterium will differ not only in chemical but also in physical properties. Therefore, it is particularly instructive to compare the properties of H₂O (water) and D₂O (heavy water) so that an explanation can be sought for many of the biological consequences of deuteration.

Investigations on physical properties between the two isotopes have been published much later (1951) by Kirshenbaum. Some of the properties of H₂O and D₂O are compared in the table below:

<table>
<thead>
<tr>
<th>Property</th>
<th>H₂O</th>
<th>D₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point °C</td>
<td>0</td>
<td>3.79</td>
</tr>
<tr>
<td>Boiling point °C</td>
<td>100</td>
<td>101.41</td>
</tr>
<tr>
<td>Density g/cc.</td>
<td>0.997074</td>
<td>1.10775</td>
</tr>
<tr>
<td>Viscosity, Millipoise (25°C)</td>
<td>8.93</td>
<td>12.00</td>
</tr>
<tr>
<td>Temperature of maximum density, °C</td>
<td>3.98</td>
<td>11.23</td>
</tr>
<tr>
<td>Ion product (25°C)</td>
<td>1 x 10⁻¹⁴</td>
<td>1.95 x 10⁻¹⁵</td>
</tr>
</tbody>
</table>

As can be seen from this table, except for the ionization ability, the values for all these properties are higher in D₂O than in H₂O. Thus,
ion product for D$_2$O is about one fifth that for H$_2$O. This change in pH essentially affects the enzyme rate specially of enzymes with well defined pH optima. Hence, to read pD from pH meter, Mikkelsen and Nielsen (1960) found out a correction factor of 0.4 which is to be added to the pH reading while reading pD. Because of higher boiling point, the fractional distillation of D$_2$O is possible. Viscosity of D$_2$O changes depending upon the temperature e.g. it will be 31% greater than that of water at 50°C and only about 13% higher at 38°C.

So far as solubility is concerned, D$_2$O is found to be a poorer solvent compared to H$_2$O. This phenomenon is again temperature dependent due to which the difference in solubility becomes less marked with an increase in temperature.

Kinetic isotope effect in metabolic studies has drawn attention of chemists and biologists as a result of isotopic replacement. In metabolic studies, if the mass of the isotopic atom involved in the reaction system differs considerably from that of abundant species of the same element, a difference in the reaction rates can be expected. This difference is said to be due to kinetic isotope effect. The latter is most pronounced among the hydrogen isotopes i.e. protium ($^1$H) and deuterium ($^2$H or D). The magnitude of this effect depends on the type of chemical reactions for which the isotope selection is effective. A series of most informative experiments have been performed on isotopic selection between H and D (Wiberg 1955, Thomson 1963). From these pioneer studies it was noticed that the reactivity of deuterated compounds is considerably lower compared to proteated ones. The important basis is the difference in reactivity between deuterium
bonds and hydrogen bonds to oxygen, carbon and nitrogen. The bonds to deuterium are more stable than bonds to hydrogen and a greater energy of activation will be required to bring the bond into activated state. Thus, at normal temperature (25°C), O-D bonds are stronger by 10.6 times than O-H bonds; C-D bonds are stronger by 6.9 times than C-H bonds and N-D bonds are stronger by 8.5 times than N-H bonds. Primary substitution of deuterium for hydrogen in a chemical bond can markedly affect the rate of scission of this bond which in turn would exert pronounced effect on the rates of chemical reactions. Therefore, the expression as ratio of the specific rate constants (kH/kD) also varies depending upon the involvement of deuterium in the reaction system. If the locus of the isotopic substitution is directly involved in the bond breaking or bond forming in the reaction, it is called a primary isotope effect (Bigeleisen 1949, 1958, Wiberg 1955). Deuterium, when located at positions in a molecule other than at the reaction locus, can also affect the reaction rate but to a lesser extent. Such an effect is said to be secondary isotope effect (Wiberg 1955).

The effect of replacement of hydrogen by deuterium in the in vivo studies has been observed by Rittenberg and Borek (1961) and Thomson (1963) mostly with enzyme reactions in animal system. Both primary and secondary isotope effects have been demonstrated depending upon the deuteration of substrate or enzyme. In dehydrogenation reactions e.g. enzymic dehydrogenation of tetra- and di-deuterated succinate, a primary isotope effect is exerted when dehydrogenation of trans-dideuterio succinate is involved (Thomson and
KLipfel 1960a). A secondary isotope effect is seen when the cis-dideuteriosuccinate is involved.

Solvent effect of deuterium oxide (D$_2$O) is of extreme importance in the biological reactions because of the differences in physical properties of H$_2$O and D$_2$O. This effect is exhibited in various processes e.g. in absorption due to higher viscosity of this solvent less amount of it will be absorbed by the organisms. D$_2$O, administered *ad libitum* in rats, caused death when 30% of the body fluid was deuterated. Enzyme reactions wherein D$_2$O is directly involved exhibit a primary isotope effect. Examples are all hydrolytic reactions and fumarase reaction in the forward direction (Thomson 1960a). One advantageous aspect of this solvent effect is the protection against radiation damage when either organisms grown in this solvent are irradiated or irradiated in the presence of this solvent. Such an effect has been reported in *E. coli* (Laser 1958), rats (Katz et al. 1957) and in maize and barley seeds (Gaur et al. 1969). Even though this effect was theoretically attributed to slower recombination rates of primary radiolysis products in D$_2$O, recent studies have proved that the degree of tissue oxygenation determines the extent of protection against radiation damage in the presence of D$_2$O.

Although deuterium occurs in nature in extremely small amounts (about 150 ppm) yet because of its utility in research and development and the availability of modern techniques it has been possible to obtain D$_2$O having almost 100% concentration. The work on its biological aspect was, however, started immediately after its discovery. That this isotope has been the principle object of study compared to all
other stable isotopes is due at least in part to the fact that substitution of $^2$H for $^1$H is the most radical possible stable isotope substitution and thus constitutes the greatest challenge to the experimenter. Another reason for this is based on economic considerations. Deuterium is the most readily available heavy isotope because of its importance in nuclear technology whereas the other heavy stable isotopes in highly enriched form especially those of biological significance have been rare and exceedingly expensive.

Most of the earlier literature concerns microorganisms, lower plants and animals. First treatise, edited by Kritchevsky (1960), emphasises on primary and secondary deuterium effects observed in a variety of microorganisms and animals. A second monograph published by Thomson (1963) and titled "Biological Effects of Deuterium" reviews the relevant work on lower plants and animals with special reference to life processes of higher animals. A subsequent report by Fläumerhaft et al (1965) summarises many observations on the biological effects of deuterium isotope with specific reference to growth and cytology of microorganisms and higher plants. Katz (1965) has also presented a comprehensive review of the effects of heavy water in both plants and animals. Later, Katz and Crespi (1971) have reported on effects of isotope in biological system but mostly covering isotope effects on chemical reactions.

Literature available on the various effects of heavy water on different biological organisms has been reviewed below in details:

Effect of heavy water on microorganisms

Availability of heavy water stimulated a great many deuterium isotope studies on the growth of considerable varieties of organisms,
xxx For the most part these studies were focused on microorganisms. This may be due to the fact that large populations with relatively well-defined biochemical properties are easily obtained. The microorganisms can be grown in a very meagre amount of D₂O. It is also true that deuterium isotope effects are by no means simple in these organisms, only organizational complexities are fewer than in higher plants and animals.

Bacteria: Earliest comment on the possible effect of deuterium on Mycobacterium tuberculosis was made by Itoh et al. (1935). An apparent stimulatory effect of intermediate levels of D₂O was evidenced from these studies. However, Lester et al. (1960) who also observed such an effect explained that physical changes produced by D₂O in the aggregation of these bacteria might cause an increased dispersion of the cells, so that there could be a greater number of colony producing particles without a real increase in cells.

Escherichia coli formed the next suitable test material for morphological studies with D₂O for several groups of investigators. Culturing of E. coli B strain in 50% (Manson et al. 1960) and in 95% D₂O (Konrad 1960) increased the lag phase and produced a progressive increase in the mass doubling time. Partial deuteration of E. coli resulted in enlarged, elongated and filamentous cells. Increase in size of the cell was presumed to be due to the inhibition of some component for cell division while cytoplasmic growth continued. Growth of E. coli was drastically inhibited in medium containing 25% D₂O (Lester et al. 1960). When E. coli K-12 strain was grown in 99% D₂O, the time lag increased
from 24 hours to 30 hours (Borek and Rittenberg 1960). They were successful in culturing *E. coli* in a medium containing 100% D₂O which resulted in a very marked change in morphology. Anomalous growth was observed when *E. coli* B and K-12 strains were cultured in agar containing H₂¹⁸O and D₂O. This anomalous growth was caused not by the rare isotope *per se* but rather by the unusual mixtures of isotopes made available to the organisms when they were transferred from the environment of one isotope to another. Subsequent studies using deuterated substrates (glucose) also had produced adverse effect on growth (Crespi *et al.* 1960b). From these studies it was concluded that microorganisms appear to have considerably greater difficulty in adapting to D₂O than in adapting to deuterio-substrates. But if the organisms can be induced to grow in D₂O with protio-substrates it is probable that replacement of the hydrogen substrate can be affected. De Giovanni and Zamenhof (1963) observed that *E. coli* grown in high concentration of D₂O contained 15.1% deuterium after 4 generations and 34.4% deuterium after 8 generations. This indicated a selectivity of incorporation of D₂O depending on length of exposures.

Simultaneously, interest of many research workers was directed in deuterating the organisms and isolating deuterated compounds. Many kinds of bacteria have been grown in fully deuterated form e.g. *E. coli* (strains B and K-12, C-600 permease- thr- leu- thiamine- and 3300 thiamine-), *Bacillus tiberrius*, *B. subtilis*, *B. cereus*, *Hemophilus influenzae*, *Serratia marcescens* and *Rhodospirillum rubrum*. Crespi and Katz (1962) could successfully isolate fully deuterated DNA from deuterated bacteria. Later, rates of reactions of deuterated enzymes acting upon protiated substrates have been studied. One such example
is the report by Rittenberg and Borek (1961) who studied succinic dehydrogenase activity both in protio- and deuterio-substrates. The reaction rates were lowered irrespective of the deuteration of enzyme or substrate.

**Fungi:** The work on fungi for deuterium isotope effects has been mostly concerned with Yeast, *Aspergillus* and *Penicillium* species. Much of the work on the effect of D₂O on yeast has been focused on fermentative and respiratory activities. A general observation was that glycolysis was depressed by about 50 per cent and respiration by 50-75 per cent in high concentrations of heavy water. When yeast cells were suspended in a medium of heavy water a remarkable and progressive inhibition of growth and respiration was observed (Lewis 1934, Taylor and Harvey 1934). Mohan *et al* (1962) sought to determine the nature of the substance required in the deuterated medium for the growth of *Torulopsis utilis*. Thiamine was found to be a growth factor and that only the thiazole moiety of thiamine was required. It was learnt from these data that deuterium blocked specially the synthesis of thiazole.

Experimental evidences by O'Brien (1963, 1964) on growth, respiration and glucose catabolism in *Saccharomyces cerevisiae* in deuterated media showed a retarding effect of D₂O on all these parameters. It was stated that deuteration of protoplasms was the primary factor in growth depression. Also, the other factor that could be attacked by D₂O substitution was the reaction rate determining factor, DNA. A block in phosphate uptake was seen in this experiment.

*Aspergillus niger* was the next interesting test material for a number of workers to study isotope effects. Crespi *et al* (1960 a) could
successfully cultivate these organisms in a medium containing 99.6% D$_2$O, deuterio-glucose and a hot-water extract of fully deuterated green algae. The growth rate was depressed but the superficial morphology of fully deuterated molds appeared normal. An improved growth rate was achieved by Katz et al. (1964) in these molds growing in a nutrient medium containing 99.6% D$_2$O, deuterio-sugars and amino acids. These experiments indicated a generalised requirement of amino acids as growth promoters for molds in D$_2$O.

Henderson and Dimming (1962) observed a synergistic effect between heat and D$_2$O. Spores of A. niger suspended in D$_2$O were more resistant to heat inactivation than those suspended in H$_2$O. From heat denaturation profiles of DNA extracted from variant strains it was postulated that heat treatment in D$_2$O caused changes in the secondary structure of DNA. In another report (Henderson and Lamonds 1966) on citric acid and pyruvate carboxylase formation by A. niger, it was suggested that the suppression of citric acid accumulation by D$_2$O could not be reversed by altering the initial pD but was possible only on transfer to H$_2$O. No apparent relationship was observed between disappearance of iso-citrate dehydrogenase activity and citric acid accumulation. On the other hand, D$_2$O grown mycelia showed pyruvate carboxylase activity only in traces. The addition of oxaloacetate, a product of pyruvate carboxylate, led to an appreciable accumulation of citric acid.

Algae: Reitz and Bonhoeffer (1935) were the first to make an investigation of the growth of algae in heavy water. Heavy water inhibits growth and higher concentrations were specially incompatible with the
growth of algae. A complete inhibition of autotrophic growth was observed by most of the workers (Weinberger and Porter 1954, Moses et al 1958, Walker and Syrett 1959). Chlorophyllous nature of algae enabled many investigators to study the photosynthesis in these organisms as affected by D₂O. Curry and Trelease (1935) reported a reduced rate of photosynthesis in Chlorella growing in D₂O medium. Craig and Trelease (1937) gave an information that at moderate and high light intensities, the rate of photosynthesis was inversely proportional to the concentrations of D₂O in the medium while low light intensities had no effect. In 1954 Horowitz studied the effect of heavy water on quinone Hill reaction of Chlorella pyrenoidosa and found that unlike photosynthesis, oxygen evolution in Hill reaction was inhibited at all light intensities. It was revealed from the studies of Moses et al (1958) that the general course of photosynthesis in Chlorella was inhibited by D₂O. From ¹⁴C O₂ uptake results of these workers it was shown that radioactivity tends to accumulate in Krebs cycle acids and amino acids, thereby demonstrating a suppression of protein synthesis.

Adaptation of algae to D₂O had naturally attracted attention of several investigators. Algae, when induced to grow in D₂O, were confronted with a difficult situation on their first being introduced to D₂O. Chorney et al (1960) noted a lengthy adaptation period required before Chlorella ellipsoidea could grow in higher D₂O concentrations. Once they got adapted to grow in this medium they would continue to grow very well and at a good rate. A drop in pH of the medium was one of the observations during adaptation. A prolonged period for adaptation might be required before growth begins and length of this period was
different with different types of algae. Chorney et al (1960), Crespi et al (1960b) and Katz et al (1964) succeeded in growing, autotrophically, a number of green algae in essentially isotopically pure $D_2O$. The so called 'monster' cells were frequently observed and were shown to contain astonishingly large amount of nucleic acids. These enlarged cells appeared to grow alright but division of cells was extremely difficult. According to Crespi et al (1960b) algae that adapted to grow in $D_2O$ did not arise from the monster cells, rather these monster cells resulted from failure to adapt. Anomaly in growth rate in *Chlorella vulgaris* was explained on the basis of some changes in the microstructure of water in isotopically heterogeneous system (Uphaus et al 1967). They also indicated that the isotopic substitution of carbon and oxygen led to far smaller effects than in the case of substitution of deuterium for hydrogen, the effect of cumulative substitution produced greater and greater deviations from normal.

The next interest in this direction of investigation was the deuteration of organisms through serial sub-culturing of them. This included replacement by deuterium not only of exchangeable hydrogen in $-OH$, $-NH_2$ and $-COOH$ groups but also of non-exchangeable C-H bonds as well (Chorney *et al* 1960, Crespi *et al* 1960b). This research was mainly aimed to obtain deuterated compounds. With deuterated compounds so obtained it was practicable to investigate the deuterium kinetic isotope effect on the slow rate of reactions in living organisms and for locating rate-controlling steps in biological systems. Quite
a number of compounds have been deuterated so far and these include proteins, nucleic acids and chloroplast pigments. Crespi et al. (1970) introduced a method to obtain hybrid proteins by enabling fully deuterated algae to utilize exogenous $^{1}$H amino acids for the biosynthesis of cellular components to form isotope hybrid proteins. These hybrid proteins are said to have considerable use in nuclear magnetic resonance studies of proteins and enzymes. In a preparative separation of deuterated protein hydrolysate by Cohen and Putter (1970), the deuterium-exchange properties of the individual amino acids were studied in relation to proton magnetic resonance. These studies suggested that all positions of amino acids were completely deuterated except:

1. the $\beta$-position of aspartic acid,
2. the $\gamma$-position of glutamic acid,
3. the C$_2$ position on the imidazole ring of histidine, and
4. the positions ortho to the hydroxyl group in tyrosine.

Thus, the microorganisms and lower plants (algae) proved useful in obtaining deuteration of physiologically important compounds. Besides, these studies yielded important information on some of the intermediate reactions of vital processes like photosynthesis. Ready adaptibility of these organisms to D$_2$O was also an outcome of these studies.

Effect of heavy water on lower animals:

Protozoa: The small size of protozoa and the ease with which one could observe the changes in them growing in heavy water media interested a few of the workers. When _Paramecium caudatum_ was put in 92% D$_2$O the
contractile vacuoles resulted into lesser frequency of contraction compared to that in water (Barnes and Gaw 1935). After 2 or 3 days these organisms even died. Circadian rhythm was shifted in Euglena gracilis when the latter were transferred to D₂O medium (Bruce and Pittendrigh 1960). In both these cases, the results reflected on metabolism of the organisms.

**Arbacia:** Cell division aspect was the next one on which many researchers have worked on, especially with eggs of sea urchin, *Arbacia punctulata.* After the studies on deuteration of cells and organisms and effects on reaction rates and metabolic processes, a sophisticated view of the role played by D₂O bonds in the three-dimensional organization of proteins and nucleic acids has been reported (Kritchevsky 1960). Deuteration was assumed to bring about disorganization of mitosis also. Antimitotic effect of heavy water treatment has been reported by a number of workers. (Cross and Spindel 1960 a,b,c, Gross and Harding 1961, Marsland and Zimmerman 1963, Zimmerman and Marsland 1964). A D₂O-induced block was found to occur at all phases of mitosis. The stabilization of achromatic figures and furrowing by D₂O attributed to sharp increase in viscosity of cytoplasm. Interference in the structure of deoxyribonucleic acid (DNA), rupture of nuclear membrane, stabilization of various gel structures like aster, spindle and peripheral plasma gel of the cytoplasm were the various observations when the test materials were immersed in D₂O. That pressure and temperature could counteract the antimitotic effect of D₂O was shown by Zimmerman and Marsland (1964), Marsland (1965) and Marsland and Austerita (1966).
Effect of heavy water on higher animals:

Most of the investigations on D\textsubscript{2}O effects on animals have been compiled in the monograph published by Thomson (1963). The first report of the effects of heavy water on a mammal was that of Lewis (1934) who gave some heavy water to a mouse which showed some signs of intoxication. Rats and mice did not show much change until about 15 per cent of body water was replaced by D\textsubscript{2}O. But once it exceeded 20 per cent level, animals became hyper-excitible, frequently convulsing and the metabolic rate would fall significantly (Katz et al 1957, Thomson 1960b). When the rats drank 50% D\textsubscript{2}O death occurred. Rats dying of deuterium poisoning had enlarged livers and adrenals (Katz et al 1957). The animals were the victims of a whole series of difficulties including kidney malfunction, anemia, central nervous damage, malnutrition and very probably other disorders. Any of these could make a substantial, if not decisive, contribution to the lethal effects of deuterium. One of the most interesting effects of deuterium in mice is a disturbance in their reproductive capacity (Hughes et al 1959, Czajka and Finkel 1960). Whether these consequences of deuteration resulted from structural changes in nucleic acids (Hughes et al 1959) or were primarily metabolic in origin, or both, remained unclear. An experiment by Bray and Thomson (1962) was carried out to determine the effect of D\textsubscript{2}O administration on incorporation of \textsuperscript{32}P into rat liver DNA. There was a progressive decrease in DNA synthesis with increasing D\textsubscript{2}O concentration. D\textsubscript{2}O administered \textit{ad libitum} to a mouse having virus-induced tumour apparently gave protection against virus which seemed to be dose dependent (Siegel 1964).
Effect of heavy water on the mechanism of energy transfer was interpreted by Kaminer (1960) from his studies on this aspect between membranes and contractible protein of frog heart. Later, extensive studies have been carried out using heavy water in the external reaction medium. On examination of oxidative phosphorylation in mitochondria it was found that oxygen consumption was decreased by D₂O although the efficiency of phosphorylation appeared to be unaffected. Oxidation was seen to suffer to a much greater extent than phosphorylation. Thus, overall effect of D₂O expressed as P/O ratio showed higher values (Laser and Slater 1960, Margolis et al 1966, Muracka and Slater 1968).

Tylor and Estabrook (1966) and Baum and Rieske (1966) observed a correlation between D₂O and other organic solvents inhibiting oxidation reactions. Kalinichenko et al (1967) found that neither D₂O nor glycerol caused significant changes in the degree of reducibility of cytochromes (b or c) in rat liver mitochondria and concluded that the viscosity of D₂O was not of fundamental importance for the respiratory depression.

Reduced rate of action of dehydrogenases in heavy water medium was reported by several workers (Thomson and Klipfel 1960a and b, Thomson et al 1962, Kahn and Rittenberg 1967, Rittenberg 1968, Henderson and Henderson 1969).

After reviewing heavy water effect on all these various systems, the reason for death occurring in deuterated animals was based on alterations in the functioning of these interrelated processes. Widespread interference in various metabolic processes in mammals seemed to be more probable due to the differential effect of deuterium on various enzymatic reactions. But as seen from available
literature enzymes isolated from rats and mice drinking different concentrations of D$_2$O were used in the understanding of mechanisms involved in the reaction rates and aimed to understand the cause for death. Investigations from Holland and Antoni (1968, 1970) suggested that heavy water would influence factor(s) regulating protein synthesis or cause changes in the conformation of certain macromolecules involved in such synthesis at microsomal level.

Effect of heavy water on higher plants:

With regard to higher plants only a few publications deal with the effect of D$_2$O and much of this work is related to germination studies. In general, these studies show that the degree of inhibition observed is reversible over relatively long periods of time upon transfer to ordinary water. Some of these reports are reviewed here. Historically, the effect of D$_2$O on seed germination has been of interest since the inhibition of the development of tobacco seeds was one of the first experiments reported on the biological effects of deuterium oxide (Lewis 1933). Germination failed to occur within two days in 100% D$_2$O and was significantly retarded in 50% D$_2$O. On transferring back to water seeds were seen to germinate after a relatively long period. A similar observation was made by Pratt and Curry (1937) for growth of wheat roots. Thus, it was seen that no higher plants can be induced to grow well in D$_2$O concentrations above 50%. Deuterium oxide (100%) was shown to inhibit germination of barley seeds but the inhibition disappeared one week after removal of seeds from D$_2$O (Von Euler 1947). It was also reported that the catalase activity
remained unaffected by D$_2$O. Ewart (1935) tried low concentrations (1-1.3%) of D$_2$O to study growth of *Lemna* and oat. While a slight stimulating effect in growth was observed in *Lemna* there was retardation in the germination of oat. In the latter case, the plants were non-chlorophyllous and had to use previously stored food materials. Some of the contradictions in the literature concerning stimulation and inhibition by D$_2$O could be resolved on the basis whether the organism had a chlorophyllous or non-chlorophyllous type of metabolism. The basis for the two different responses has, however, been not given by these workers. Wheat embryos, devoid of endosperm, when cultured in 14.8 to 94% D$_2$O and ordinary water, showed no difference in rate of respiration for the first two days of germination (Melot 1934). Brun and Tronstad (1935) reported that peas (*Pisum sativum*) germinate in 40% D$_2$O but are completely inhibited at 50%. A study was made of the toxic effect of heavy water (D$_2$O) on the sprouting of seeds and on the enzymatic activity of plants by Badanova (1956). He observed that seeds containing different stored substances were characterised by different resistances to D$_2$O. In this manner, oily seeds of sunflower were most resistant ones while starchy seeds like wheat occupied intermediate position and proteinaceous seeds like peas were least resistant.

Galonska and Hubner (1963) used cylinders of *Agrostemma* hypocotyl and *Avena* coleoptile as test materials for their growth in heavy water. Growth in length was completely inhibited in both test specimens by D$_2$O. These results indicated that the inhibition by D$_2$O could be attributed to the influence on the perception of the growth.
stimulus by plant. Stein and Forrester (1963, 1964) examined the effect of high concentrations (80-90\%) of \( \text{D}_2\text{O} \) on the roots of peas and corn (\textit{Zea mays}) by dipping them in heavy water. A bulge, appearing just above the meristematic region of the roots, was histologically observed to be due to the radical enlargement of cortical cells. \( \text{D}_2\text{O} \) was seen not to prevent initiation of laterals in pea roots but affected their ability to function. Results of this report suggested that monocots seemed to be more sensitive to \( \text{D}_2\text{O} \). Among the many effects observed as a result of treatment with \( \text{D}_2\text{O} \) are included changes in cell shape, staining intensity, cessation of mitosis, etc. It was Stein and Forrester (1964) who first brought in the term 'adaptation' in higher plants as connected with \( \text{D}_2\text{O} \). Their experiments included the step adapting system i.e. by pretreating in lower concentrations and then growing in higher concentrations of \( \text{D}_2\text{O} \). This pretreatment for longer periods was assumed to equilibrate the tissue with \( \text{D}_2\text{O} \). According to them adaptation must involve metabolic processes which become very slow or some structural changes of the chemical apparatus of the cell. The differences in reaction rates had been explained on the basis of \( \text{D}_2\text{O} \) causing an imbalance of reactions.

Work on mint (\textit{Mentha piperita}) by Blake \textit{et al} (1964a) appears to be the most detailed study on the \( \text{D}_2\text{O} \) effect on higher plant. They grew the plants in varying concentrations of \( \text{D}_2\text{O} \) over a period of at least 50 days. Extensive replacement of hydrogen by deuterium caused a reduction in shoot elongation, leaf blades and petioles. Leaves from these plants remained epinastic and eventually turned necrotic, brown and dry. A second report from this group of workers was mostly concerned
with leaf and stem histology (Grane et al. 1964). Comparative microphotographs of leaf blade, stem and root from control and D$_2$O treated plants showed marked difference in cell shape and size, cortex region and vascular bundles. From these studies, they concluded that D$_2$O exerted its effect by way of impairment of cell division, presumably as a result of disturbed enzymatically controlled biosynthetic reactions. D$_2$O effect was seen more markedly in differentiating tissues than in those already differentiated. In another report, Blake et al. (1964b) showed the effect of certain growth regulators on mint plants along with D$_2$O in the nutrient solution. The growth substances like GA, NAA and IAA stimulated growth in the presence of D$_2$O. The most interesting and significant observation was that low concentration of malic hydrazide (MH), normally inhibitory of vegetative growth, stimulated growth of D$_2$O treated plants. While investigating environmental stress factors in seed germination and seedling growth, Siegel et al. (1964) had shown that Winter rye seeds were found to be uniquely D$_2$O resistant. Cope et al. (1965) described in detail the effect of $\text{H}_2\text{O}$ on higher aquatic plant species namely, Lemma gibba, L. verruca and L. minor (duck weed). They had shown that these plants proved refractory to culture in high concentration of D$_2$O. Lemma seemed to be unable to adjust to any concentration of D$_2$O above 63%. The abnormalities induced by treatment were found to be eliminated to a considerably extent by the addition of kinetin to the nutrient medium. But kinetin effect was nullified by D$_2$O above 63%. Studies on Atropa belladona (Uphaus et al. 1965) had shown that deuterium oxide (50%) decreased alkaloid content progressively. Fruit size was reduced and the viability of seeds obtained from these plants was markedly less. When Arabidopsis thaliana seeds were grown in different D$_2$O
concentrations ranging from 0 to 50%. Germination and appearance of leaf pairs were delayed proportionate to D$_2$O concentrations (Bhandarkar and Gaur 1967, 1968, 1970). In 50% D$_2$O, growth stopped completely after 3rd pair of leaves. Seed weight remained same in control and treated, but the number of seeds per plant was markedly reduced. Seeds thus obtained were again grown in the respective concentrations of D$_2$O (as in the F$_1$ generation). They showed further inhibition in germination rates and ontogenic development. Gibberellic acid at 2.5 x 10$^{-5}$M concentration not only assured 100% germination but also it hastened germination in 50% D$_2$O. Experiments on physiological effects of heavy water on absorption by Bhattacharya et al (1969) revealed a differential absorption in seeds with different reserve food materials like starch (barley and rice ...), fat (linseed and mustard) and protein (mung and kidney beans). The profile of initial hydration was different in the proteinaceous seeds compared to other starchy and fatty seeds. Respiration rate was also low in D$_2$O treated seeds while a higher RQ was obtained for these seeds.

Recently, Waber and Sakai (1974 and 1975) reported that ultrastructure of cells of plants grown in D$_2$O (99.8%) and H$_2$O did not differ much although differences were observed in chloroplast, dictyosome morphology and ribosome number. This comparison was made between 2 day old water grown plants and 9 day old D$_2$O grown plants because of their morphological similarity at the two different chronological ages. Uphaus et al (1975) studied effect of increasing concentrations of heavy water (0 to 70%) on growth, development, morphology and transpiration in Nicotiana tabacum. Marked reduction in size of plant, alkaloid production and flowering, etc were some of the deleterious effects of
D₂O treatment. Transpiration data showed that total liquid uptake (and thus the total water transpired) decreased with increasing levels of tissue deuteration. Finally, having evidenced effect at every level of plant organization, they viewed deuterium oxide as a non-specific chaotropic agent.

SCOPE OF WORK

From the foregoing literature review it is apparent that substitution of hydrogen by deuterium represents an environmental alteration that the organism must be able to contend with if it is to survive. Thus, replacement of biologically important isotope (like hydrogen) by the corresponding heavier stable isotope (deuterium) has been seen to bring about marked morphological, cytological and physiological changes in the cells and tissues of both lower and higher plants and animals. In view of increasing interest in the problem of deuterium isotope, especially on higher plant growth, this problem has been explored in the present studies. In our experiments, barley seeds (Hordeum vulgare) serve as test material, and deuterium oxide (heavy water) forms a growth medium. A differential absorption of H₂O and D₂O by seeds is expected to influence the activation and synthesis of certain enzymes responsible to trigger various metabolic processes. Interference by D₂O, in any phase of mitotic activity, increased viscosity of cytoplasmic gel, stabilization of achromatic figures and forrowing together would disturb the geometry of cell division.
The studies regarding more sophisticated view of the role played by change in D₂O bonds such as O-H, O-D, C-H, C-D; and N-H and N-D, in three dimensional organization of proteins and nucleic acids have shown D₂O to be a physiological tool of great value. Because of this substitution of deuterium for protium the enzyme reactions can also be retarded which, in turn, will reflect on the organism at physiological and morphological levels.

Based on some of these generalizations and specially due to the marked paucity of detailed studies on the D₂O effects on higher plants we are encouraged to undertake the research described in various sections of this thesis. Section II on morphological and histological studies includes various measurements of length and weight of different parts of seedling and also histology of leaf whir! of barley seedling. Recorded in the next section, the physiological aspects included the effect of D₂O on the changes in the food reserves and their translocation to growing axis. These studies included changes in various types of barley proteins and amino acids during seedling growth. Protease activity measurement is an indication of catabolic capacity of enzyme while label incorporation studies of protein precursors express the protein synthesising capacity in D₂O grown plants. Under carbohydrate metabolism, - and - amylase activities are measured and influence of GA concentrations on enzyme formation is studied in half seeds (Section IV). Since these processes in the preceding sections are energy dependent, any impairment in energy metabolism due to D₂O can also disturb the plant growth. Hence, these studies were undertaken and described in Section V.

An interlinked story on the "Effect of heavy water (D₂O) on physiological processes in barley (Hordeum vulgare)" is presented in a self-contained manner with regard to the basis, observations and discussions of results.