Discussion
Germination is a growth process of the embryo characterized by a rapid uptake of water. This initial uptake of water appears to be a dominant factor in inducing the germination process. The nature of uptake and the sequence of events initiated by the entry of water are complex. Among the interesting biochemical phenomena are the degradation of seed reserves, synthesis of new plant materials, a changing array of enzymes and of new and novel metabolities. Food reserves in storage organs are mobilized during seed germination and post-germinative growth of seedlings. Correlations, derived mainly from time-course studies, between the decline in reserve materials and growth of embryonic axis indicate that hydrolytic products are translocated to the growing axis.

In most of the oil seeds the radicle emerges through seed coat within 24 h after imbibition. As the seed imbibes water, hydration of cells takes place resulting in cell expansion and increase in cell size. By utilizing the reserve food in the cotyledons, through the metabolic activities, the radicle and hypocotyl develop from the embryo of the seed.

The dry seed is characterised by a remarkably low rate of metabolism. Seeds germinate when favourable conditions are provided especially adequate supply of water. As the seed is hydrated marked changes in its
various parts occur. The imbibition of water results in the hydration of cells leading to cell expansion and increase in cell size. The size, mucilage content and nature of seeds appear to have marked influences on germination. Many of the seeds placed in petri dishes under optimal conditions for germination show a triphasic pattern of water uptake. Initial uptake of water in Phase I (imbibition) followed by a lag period of water uptake and finally in a Phase III in which the hydration is associated with visible germination. The length of each of these phases depends upon certain inherent properties of the seed and upon conditions to exposure to water (Bewley and Black, 1978). In the present study the increasing variations in fresh weight of cotyledons of safflower seeds during the eight day germination period appear to result from continued uptake of water due to increase in cell size and cell expansion.

The hydration of the seed leads to a series of chemical changes which are complex in nature, the foremost among them appear to be degradation of stored materials to meet the requirements of growing parts. The changes in fresh and dry weights of the cotyledons of safflower seeds during germination (Fig. 1) in the present study is consistent with the earlier observations in castor bean seeds
Seeds, which store lipid, mobilize their oil stores during germination. Nearly all of the lipid and other reserves in oil rich seeds are mobilized after emergence of radicle like in castor bean (Marriott and Northcote, 1975), soybean (Yashida, 1984) and sunflower (Bose et al., 1988). It was reported that in germinating castor bean (Younis et al., 1971) and cucurbita pepo (Thomas and Rees, 1972), the decrease of total lipid content may be due to its conversion into carbohydrates which was confirmed by the increased levels of carbohydrates. In the present study also decline in fat is accompanied by the increased formation of carbohydrates (Figs. 2 and 7).

The fall in the total lipid levels in the cotyledons of germinating safflower seeds (Figs. 2 and 3) does not run parallel with the fall in triacylglycerols, apparently due to the increased formation of phospholipids. Similar observations were noted in germinating hazel seeds (Shevery et al., 1973) in castor bean (Marriott and Northcote, 1975), in soybean (Harwood, 1975), and in sunflower seeds (Bose et al., 1988). Phospholipids are necessary for membrane biogenesis and in the synthesis of newer cells and organelles during germination process (Marriott and Northcote, 1975). In the present study also increased formation of phospholipid
were observed in safflower (Fig. 4). The proliferation of cells and increase in the population of glyoxysomes and mitochondria during germination of different seeds lends support to the changes in phospholipids (Marriott and Northcote, 1975; Bose et al., 1988).

Breakdown of fat or oil reserves involves the participation of 3 discrete bodies. These are the fat storing oil body, the mitochondria and the glyoxysome. These three organelles function as follows: (1) lipolysis to give fatty acids and glycerol occurs in the oil bodies; (2) oxidation of fatty acids and synthesis of succinate via the glyoxylate cycle takes place in the glyoxysome; and (3) conversion of succinate to oxaloacetate by the mitochondria. The oxaloacetate is processed further in cytoplasm to yield sucrose (Bewley and Black, 1978; Murray, 1984).

Several lipases have been demonstrated in seeds, which differ in pH optimum for their activity. An acid lipase generally appears first and seems to be associated with spherosome, while an alkaline lipase appears later and is largely associated with glyoxysomes (Stumpf and Broadbeer, 1959; Muto and Beevers, 1974; Mayer and Shain, 1974; Anthony et al., 1978; Murray, 1984).

Seed lipases are generally non-specific and can
hydrolyze a wide variety of triglycerides (Bewley and Black, 1978). However, the triglycerides are not necessarily broken down uniformly and the fatty acids may be released selectively. The lipase from Brassica compatris attack triolien more slowly than either triacetin and and tributyrin (Wetter, 1957). In seeds of cotton linoletic and palmitic acid are broken down preferentially compared to stearic and oleic acids (Joshi and Doctor, 1975). The existence of lipase with an optimum pH of 8.0 in safflower seeds has been demonstrated in the present study (Fig. 10). The development of lipase (Fig. 11) in these cotyledons resembles the situation observed in peanut (Jacks et al. 1967), Douglas fir (Ching, 1968), Leptospira pomona (Berner and Hammond, 1970), Wheat (Tavenger and Laidman, 1972), Apple (Smolenska and Lewak, 1974) and in Cucumis (Opute, 1975).

Although earlier measurements of lipase activity in different seeds used synthetic substrates, no study has been carried out so far using native triglycerides extracted from the same seed. Lipase extracted from safflower seeds is capable of hydrolyzing native triglycerides, although at a lesser rate compared to the hydrolysis of olive oil (Fig. 11). Nevertheless the development of lipase activity in the cotyledons of germinating safflower seeds is sufficient to account for the observed degradation of stored oil.
As indicated in Figs. 11 and 2, there is good correlation of lipase activity and disappearance of lipid in the cotyledons of germinating safflower seeds, substantial changes occurring between day 2 to day 6. In castor bean, mobilization of stored lipid begins on about the third day after imbibition and digestion is completed on seventh day (Slack et al., 1977). During the eleven day period after imbibition of peanut a decrease in fat content of 55% representing 9.4 \( \mu \) moles of triglycerides/cotyledon/day was noted (Jacks et al., 1967).

In many fat storing seeds free fatty acids liberated by lipolysis do not accumulate but are rapidly degraded further and converted to carbohydrates. The glyoxylate cycle has been shown to operate in many seeds for example in Arachis and Ricinus (Kornberg and Beevers, 1957; Marcus and Velasco, 1960; Yamamoto and Beevers, 1960; Mettler and Beevers, 1980). In the present study the development of isocitrate lyase activity (Fig. 16) in the cotyledons of germinating safflower seeds lends support to the development of glyoxylate cycle in safflower seeds. The level of isocitrate lyase in dry seeds is very low or absent in some seeds (Lado et al., 1968; Singh et al., 1981).
In the present study the level of isocitrate lyase in dry safflower seeds is extremely low, raises very rapidly on germination reaching peak by day 4 and declines further with the level being nearly 10 fold higher by day 8 coinciding with the development of lipase activity and degradation of lipids and synthesis of carbohydrates (Figs. 2, 3, 7-9 and 11).

Regulation of metabolic reactions can be achieved through control of enzyme activity by factors including substrate and product concentration and co-factor concentration, other metabolites and by a change in the amount or concentration of an enzyme. Studies with peanut cotyledons (Gientka and Cherry, 1968), squash seeds (Penner and Ashton, 1967), soybeans (Singh et al., 1981), castor bean (Lado et al., 1968), and cucumis (Slack et al., 1977) have shown de novo synthesis of isocitrate lyase at that time when the enzyme is required to participate in the glyoxylate cycle and the control on the development of this enzyme is exerted by the hormones of the embryonic axis (Penner and Ashton, 1967; Gientka and Cherry, 1968; Slack et al., 1977).

The products of triacylglycerol degradation may be utilized for further fat and membrane production. However,
a major proportion of the breakdown products are converted finally to sucrose. Several reports indicated that the decrease of fat reserve with concomitant increase in the levels of carbohydrates during germination of several oil seeds (White, 1958; Thomas and Rees, 1972; Bhatia et al., 1978).

Invertases play effective role in sucrose metabolism in germinating seeds. Development of acid and alkaline invertases was observed in carrot (Ricardo and Rees, 1970), maize (Tsai et al., 1970), mung bean (Dey, 1986) and horsegram (Karunagaran, 1990). The presence and variation in the activities of acid and alkaline invertases in germinating safflower cotyledons, observed in the present study (Fig. 12) suggests that the cotyledons may actively synthesize sucrose, a part of which may be hydrolyzed therein by invertase and a part may be translocated to growing axis for utilization.

The developmental profiles of the invertases during the eight day germination period suggests that acid invertase may be intimately connected with hydrolysis of sucrose, as the degradation of triglycerides and the increase in reducing sugars coincide with the development of acid enzyme rather than alkaline invertase (Figs. 13 and 14). Similar
patterns of invertase development have been shown in *cicer arietinum* (Azhar, 1972), mung bean (Dey, 1986) and horsegram (Karunagaran, 1990) involving de novo synthesis. With the increased availability of reducing sugars, the cotyledons of germinating safflower seeds appear to synthesize starch and non-reducing carbohydrates required for cellular growth and multiplication.

Part of the pathway of the gluconeogenesis is the reverse of glycolytic sequence. Glycolysis appears to be a universal feature of tissues of higher plants (Thomas and Rees, 1972; Ching, 1972; Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982; Murray, 1984). This poses a question of the relationship between gluconeogenesis and glycolysis during the germination of fatty seeds. Although studies have been carried out with castor bean (Yamada, 1955) and *cucurbita pepo* (Thomas and Rees, 1972) further studies are required with different oil seeds on the regulation of these two pathways as the relative activity of the two pathways could profoundly affect the utilization of storage fat.