PHYSIOLOGICAL CHANGES IN DEVELOPING PEA SEEDS

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ABSTRACT

Physiological changes in developing pea seeds have been investigated in relation to the development of the ability of seeds to withstand desiccation and to germinate as succulent seeds when taken directly from the pod. In the developing garden pea seed the ability to withstand desiccation was found to be preceded by a fall in respiration rate and seed moisture content, both of which followed a sharp decline in ethanol soluble sugars. When harvested seeds were kept in high humidity conditions respiration was found to decline even though the moisture content was maintained. The fall in respiration was always associated with a fall in the level of sugars and seeds whose respiration rate had fallen in humid storage could be induced to respire more rapidly by the addition of sucrose. Further, in defoliated plants kept in the dark the sugar content of the seeds still attached to the plant was reduced resulting in a reduced respiration rate. It is suggested that seeds can only withstand rapid desiccation after a decrease in physiological activity assessed by O_2 uptake, following a fall in the supply of respiratory substrate in the form of sugars.

During development, the ability of seeds, desiccated over calcium chloride under vacuum, to retain solutes improved with age and with storage under high humidity conditions. This finding was investigated further by looking at the changes in phospholipid levels of succulent seeds during normal development and after three days storage under 100% rh. It appeared that improved solute retention was associated with an increase in the phosphatidyl choline levels in succulent seeds before they were dried. The

desiccated seed on the other hand was characterised by a loss of phospholipid phosphorus and by an increase in the number of unidentified phospholipids as compared with undried seeds of the same age.

The effects of the respiratory inhibitors potassium cyanide and salicylhydroxamic acid were examined at various stages of development. It was concluded that the TCA-terminal oxidase system is the main respiratory pathway in developing pea seeds. During the time period studied there appeared to be no transition from one aerobic respiratory pathway to another.

During the course of this study it was noticed that immature seeds were capable of germinating before developing the ability to withstand desiccation. When immature succulent seeds were set to germinate, germination percentage was improved by the removal of the testa; however the early growth of immature seeds remained low but increased with age. It is suggested that premature germination of seed is prevented by a combination of a reduction in O_2 supply and the strength of the testa. The low vigour of immature seeds is not influenced by these factors but by the poor ability of seeds to mobilize their storage reserves.

ABBREVIATIONS

DAFB	Days after full bloom
s.t.p.	Standard temperature and pressure
v/v	Volume by volume
w/v	Weight by volume
rh	Relative humidity
O_2	Oxygen
CO_2	Carbon Dioxide
KCN	Potassium cyanide
SHAM	Salicylhydroxamic Acid
ABA	Abscisic Acid
MCIA	Methyl-4-chloroindol-3yl acetate
PA	Phosphatidic acid
PC	Phosphatidyl choline
PI	Phosphatidyl inositol
PE	Phosphatidyl ethanolamine
PG	Phosphatidyl glycerol

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INTRODUCTION

In both the animal and plant kingdoms one of the most conspicuous evolutionary themes has been the increasing protection of the young against hazards inherent in the colonisation of a terrestrial environment (Good, 1956). The evolutionary outcome of this theme can be seen in the placental habit adopted by the Angiosperms and the Placental Mammals (Good, 1956; Harper, Lovell and Moore, 1970), which provides maternal nutrition and protection for the developing embryo.

In the Angiosperms successful reproduction depends on the integration of four stages of the life cycle; fertilization, seed development, seed dispersal and seedling establishment. During seed development the placental habit ensures protection of the embryo from dehydration, provides nutrition throughout its development and facilitates seedling establishment by the provision of storage reserves. Once matured, the seed is dispersed away from its sedentary parent and a wide variety of morphological adaptations have evolved to assist dispersal (Stebbins, 1971). Annual species have, for the most part, evolved in environments which have had seasonal variations in conditions including the existence of an unfavourable season. Stages of the life cycle of annual species are coordinated to ensure that germination and seedling establishment take place after dispersal only in conditions favourable to seedling growth. As a result, in environments that in one year have both favourable and unfavourable conditions, plants have evolved seasonal cycles of reproduction with maximal growth of the parent plant under the most favourable conditions,

resulting in seed production before adverse conditions are encountered. Thus the seed habit has evolved as a major means of surviving hostile environments.

The outstanding characteristic of seeds of plants that have evolved under seasonal pressures is their desiccated condition. In such a state the seed uses very little of its reserve energy for maintenance and is resistant to such adverse conditions as drought and cold. The main emphasis in the evolution of reproductive morphology has been the prevention of extreme water fluctuations which might lead to death. In the Angiosperms maternal protection of the developing seed ensures that the seed is not subjected to drastic changes in moisture content until it has developed to a stage when it is resistant to desiccation.

The evolution of a desiccated seed capable of withstanding unfavourable conditions while containing sufficient food reserves for the establishment of a seedling once conditions are favourable has been one factor responsible for the success of the Angiosperms. The appearance of seeds with a relatively high nutrient content has had an important impact on the evolution of Man. Angiosperm seeds have been and still are Mankinds most important source of food. This was of critical importance to Man in the change from a nomadic hunting to a sedentary farming existence. Since Neolithic times Man has cultivated various plants, the most important being the cereals such as barley and wheat and the legumes such as beans, peas and lentils.

One of the four most important legume crops in the world today

is peas with a world production of about 10 million tons (Davies 1976). Peas belong to the genus Pisum and the most commonly grown species in the British Isles is P. sativum which originated in the drier habitats of the Near East. Carbonised seeds dating from 7000 B.C. which have been found in N.E. Iraq and S.E. Turkey provide the earliest evidence of peas. These seeds were thought to have already been cultivated (Zohary and Hopf, 1973). The wild ancestoral species of the cultivated pea are still unknown but cultivated pea seeds are believed to have spread from the Near East westwards towards Europe (during the Bronze Age) and eastwards to India via the Himalayas and Tibet to China (Brouk, 1975). The pea crop has been cultivated from such early times, not only for its dietary value but also for its ability to improve soils (Bland, 1971). Up until about the last twenty years only the mature dried seed was used as a vegetable but with the development of the freezing industry a demand has arisen for the sweeter wrinkle-seeded cultivars of peas.

Pea seeds have been chosen as the experimental material for this thesis not only because they are an important source of food but also because they represent a good example of an annual Angiosperm. The general observation, made earlier in this Introduction, that Angiosperm seeds can exhibit the phenomenon of drying to low moisture levels without harmful effects on their subsequent germination, is well exemplified by the pea seed which also provides a convenient model system of a "typical" dicotyledonous plant for the investigation of this phenomenon. Leguminous seeds as a whole, provide good model systems of a "typical" dicotyledon and as such many investigations have been carried out using different legumes.

Changes occurring in the leguminous seed during development from fertilization up to full maturity have been well documented. Essentially the observations that have been made can be divided into two types; physiological processes covering anything from a change in weight to a change in D.N.A. content of the seed and ultrastructural changes during development some of which can be related to specific physiological processes.

Of the many physiological processes occurring during development, changes in weight are perhaps the most easily measured. Changes in both the dry and fresh weight of developing leguminous seeds have been recorded with the difference between the two values being regarded as a measure of the moisture content of the seed. During the development of soybeans (Bils and Howell, 1963), lima beans (Klein and Pollock, 1968), dwarf beans (Opik, 1968) and castor beans (Marré, 1967) the dry weight of the seeds has been shown to increase throughout the period studied while the fresh weight and the moisture content of the seed increased early in development but later declined to low levels. A similar pattern of changes in dry weight, fresh weight and moisture content have been found in developing pea seeds by Bisson and Jones (1932), Turner, Turner and Lee (1957), Bain and Mercer (1965), Matthews (1973a) and Millerd and Spencer (1974). Although changes in the moisture content of a developing seed are of interest in that, at full maturity the seed is a resistant, viable structure with a low moisture content, there are other attributes shown by a mature seed. The leguminous seed like many other reproductive structures is, at maturity, an independent entity containing storage reserves in the cotyledons for maintenance of the embryo and establishment

of the young seedling. In the case of crop plants grown for their seed, these reserves have been exaggerated by selection. In castor beans the main storage reserve is oil and Marré (1967) has shown that this increases throughout the development of the seed. In peas, the major storage reserve is starch (Bisson and Jones, 1932; McKee, Robertson and Lee, 1955; Danielson, 1956 and Bain and Mercer, 1966) which also increases throughout development. Although there is a general tendency for the major storage reserve to increase throughout development there is some variation between closely related legumes, for example, in P. sativum starch tends to increase throughout development whereas in P. arvense the period of starch synthesis is much reduced such that during the later stages of development the starch content levels out (Flinn and Pate, 1968). Starch is not the only carbohydrate found in developing seeds. In pea seeds (P. sativum) soluble sugars are found in increasing amounts early in development but fall to low levels at full maturity (Bisson and Jones, 1932; McKee et al, 1955; Bain and Mercer, 1965). The major soluble carbohydrate found in pea seeds is sucrose (Turner et al, 1957) which makes up about 95% of all soluble carbohydrates (Davidson, 1956).

The other major storage reserves found in leguminous seeds are proteins and these too have been found to increase throughout development. In dwarf beans (Opik, 1968) total nitrogen increases throughout while in peas both the total nitrogen (Bisson and Jones, 1932; Bain and Mercer, 1966) and the protein nitrogen (McKee et al, 1955; Millerd and Spencer, 1974) increase throughout. Soluble nitrogen on the other hand, follows a similar pattern to that of soluble carbohydrates, rising early in development then falling to low levels at maturity (McKee et al, 1955; Flinn and Pate, 1968).

Many other chemical compounds show changes in levels during seed development. Phosphorus is found as a storage reserve in the form of phytin (Gontzea and Sutzescu, 1968; Guardiola and Sutcliffe, 1971) and, because of its importance in both phosphorylation and membrane metabolism it is also found in forms not associated with storage reserves. Throughout the development of pea seeds total phosphorus increases (McKee et al, 1955; Rowan and Turner, 1957). Other biochemical pathways essential to any physiological processes are those of RNA and DNA synthesis; these are the basis of any protein synthesis be it for storage or for the control of physiological processes such as carbohydrate metabolism. Again these show patterns of change throughout development however, unlike phosphorus, both RNA and DNA increase early in development subsequently falling to low levels at full maturity in pea seeds (Millerd and Spencer, 1974).

Not only is the leguminous seed at maturity a resistant independent entity with a low moisture content and sufficient reserves for maintenance and growth of the embryo but at the same time it has a very low level of physiological activity. The physiological activity of seeds has, most commonly, been estimated by using respiratory activity as an indicator and in the changes leading to a mature dry seed a general decline in physiological activity has been observed. When O_2 uptake was expressed on a per seed basis in developing soybeans (Bils and Howell, 1963; Ohmura and Howell, 1962), castor beans (Marré, 1967) and dwarf beans (Opik, 1968) the respiration rate first rose then fell to a level at full maturity which was barely measurable. This pattern of change in respiratory activity is also found in developing pea seeds (McKee et al, 1955; Kollöffel, 1970a, b; Matthews, 1973a).

2 PAGES

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Thus during development the pea seed changes from a structure dependent on its parent for supplies of water, carbon and nitrogen (Pate, Sharkey and Atkins, 1977) to an independent entity capable of becoming a new individual. In doing so, it changes from a structure showing high levels of moisture, soluble carbohydrates, soluble nitrogen, DNA, RNA and a generally high level of physiological activity measured by respiration rate per seed to a structure low in physiological activity, moisture, soluble carbohydrates, soluble nitrogen, DNA and RNA but high in storage reserves such as starch, proteins and phytin. The increase in dry weight of the seed has been attributed to the increase in these storage reserves but what changes occur during development which lead to the achievement of a state of low physiological activity remains unresolved. Certainly, such physiological changes as fresh weight, moisture content, soluble carbohydrate, soluble nitrogen, DNA, RNA and respiration rate all show a similar pattern of changes during development, in that early in development they increase to a peak subsequently declining to the low levels characteristic of the mature seed. However, what causes this switch to declining rather than increasing levels is not known and when, in physiological terms the seed first develops the ability to withstand a drastic fall in moisture remains uncertain.

Observations have been made on ultrastructural changes occurring during the development of leguminous seeds. Early in the development of soybeans (Bils and Howell, 1963), dwarf beans (Opik, 1968) and lima beans (Klein and Pollock, 1968) there is the formation of cells with a full complement of organelles and with a full vacuolar system. As development proceeds there is an increase in storage reserves seen as an increase in plastids such as

amyloplasts and protein bodies but there is also a tendency for other cellular structures to be degraded. Thus, at full maturity the golgi have tended to disappear, the mitochondria and other membranes are less distinct, the polysomes have become degraded, the endoplasmic reticulum has become reduced while lipid vesicles have appeared and are characteristically orientated around the plastids and under the cell wall.

Bain and Mercer (1966) have found that similar changes occur in developing pea seeds which they related to the physiological events that occur during development. In this way they illustrated that development consists of an interrelated series of events. In their study they distinguished four phases in the development of garden pea seeds (cv. Victory Freezer) each characterised by different metabolic events. The first phase was characterised by active cell division in which the embryo became differentiated, the second by cell expansion resulting in growth of the embryo, the third by synthesis of storage reserves leading to an increase in the dry weight of the embryo and a final phase, termed maturation, during which the embryo becomes dehydrated and a dry but viable seed is produced. It is in this final phase that the embryo develops the ability to withstand an extremely low moisture content.

Although physiological and ultrastructural changes have been observed to take place in developing seeds, little work has been done on how these changes are related to the development of the ability of mature seeds to withstand desiccation. The coincidence between the fall in respiration rate (Klein and Pollock, 1968; Marré, 1967; Kollöffel, 1970a; Matthews, 1973a) and the fall in seed moisture content during the final phase of seed development

has resulted in the suggestion that a gradual increase in the water stress of the seed may be partially responsible for the decrease in respiration rate and hence in physiological activity. This finds support in the association between respiration rate and water content in the germinating seed (Opik and Simon, 1963; Marré, 1967) where respiration rate and water content show parallel increases. A similar phenomenon has been shown in the initial rise and subsequent fall in respiration and enzymic activity that has been found in water stressed organs such as wilting leaves (Vaadia, Raney and Hagen, 1961; Crafts 1966). However, it has been suggested that changes in moisture content are themselves linked to changes in sugar content (Bisson and Jones, 1932; Bain and Mercer, 1966) thus many events are taking place at approximately the same time during development and it may well be that the stimulus for both a decline in physiological activity and the subsequent development of the ability to withstand desiccation in pea seeds is not simply mild water stress.

One of the other major sequences of physiological events occurring in developing pea seeds are those of carbohydrate metabolism. These have been the subject of much work because of their importance to the final food quality of the seed (Bisson and Jones, 1932; Turner et al., 1957; Danielson, 1956). The main soluble sugar found in pea seeds is sucrose (Turner et al., 1957). It has been suggested that during phases 2 and 3 of development the major function of sucrose was that of controlling the osmotic pressure of the vacuoles of the embryonic cells thus providing the necessary turgor pressure for cell expansion but that later on, in phase 4 sucrose becomes available for starch synthesis thus declining to low levels (Bain and Mercer, 1966). At the same time as

this decline in sucrose levels there is a decline in both moisture content and respiration rate and further, the seed first develops the ability to withstand desiccation. This raises the possibility of the involvement of carbohydrate metabolism with physiological activity.

Physiological activity, as has already been said, has been measured using O_2 uptake (Kolloffel 1970a, b; Matthews, 1973a) however, although O_2 uptake is a measure of aerobic respiration, there is some evidence that this is not the only respiratory pathway used in both developing and germinating seeds. Wager (1957) has shown in developing pea seeds that at full maturity the rate of aerobic respiration in pea seeds has fallen by half but that the rate of anaerobic respiration has altered little. Further, in a review Goddard and Meeuse (1950) have suggested that early in development the cytochrome oxidase system found in the TCA cycle was predominant but that later polyphenol oxidase became the more normal terminal oxidase. Work on germinating pea seeds by Yentur and Leopold (1976) suggested that an 'alternate cyanide-insensitive pathway' was the main respiratory pathway in use during the first 5 to 8h but that after 8h the TCA cycle gradually increases in significance. Roberts (1969) has also postulated this shift from one respiratory pathway to another in germination. He has suggested that early respiration in germination is via the Pentose Phosphate Pathway but that later in germination the EMP pathway becomes predominant.

This idea of the possibility of more than one respiratory pathway being involved in germination is further supported by many steps in the TCA and the Pentose Phosphate cycles having been worked

out in rehydrated, hence germinating pea seeds. However, relatively little is known about the respiratory systems in developing seeds, especially in relation to the ability of mature seeds to withstand desiccation. It is tempting to postulate that if there is sequential activation of respiratory systems once germination starts then there might be sequential deactivation of respiratory systems in developing seeds. In this context Marré (1967) in a reviw on the development and germination of castor beans and Kolloffel (1970a, b, c) in peas have shown that changes other than gross 0_2 changes do occur, for example, respiratory control and phosphorylation activity decrease during development but increase again during germination while Thomas (1972) has suggested that desiccation resistant respiratory systems may be 'selected' for during development in preparation for total inactivation in the dry seed. What changes occur in the respiratory metabolism of developing pea seeds and what relation these might have to the development of the ability to withstand desiccation have not so far been investigated.

It has already been stated that seed development is the result of the interrelation of many events including changes on the ultrastructural level. It has been noted in several species that during dehydration one of the most marked changes has been the increasing lack of distinction of cellular membranes as development proceeds to maturity (Bain and Mercer, 1966; Klein and Pollock 1968; Payne and Boulter, 1969).

These changes in the membrane structures of the seed could have drastic effects on the metabolic processes of the cell, for example, Kolloffel (1967; 1970b) has shown that enzymes of the electron transfer chain in pea seeds are localised on the inner

mitochondrial membranes. Thus, membranes can be considered to have a central role to play in the subcellular organisation of the cell. They may control metabolism in several ways, by passive separation of the functionally different parts of cells; by actively controlling the movement of metabolites; by possessing specific groupings of enzymes and by having specific metabolic properties (Bain and Mercer, 1966; Van Deenan 1966; Koostra and Harrington, 1969; Van Deenan, Degier and Demel 1972; Kates and Marshall, 1975).

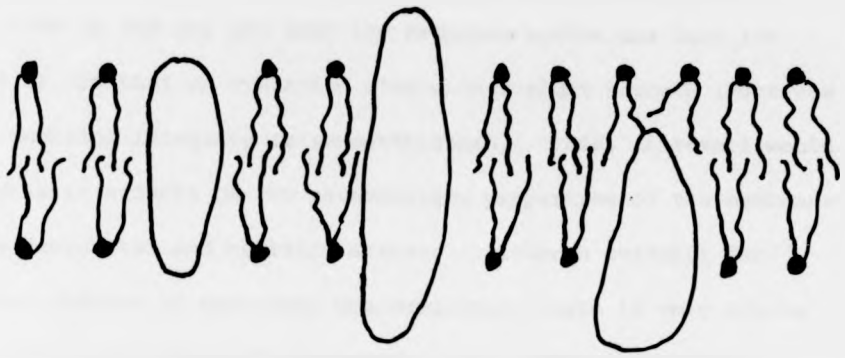
If membranes have such a central role to play in not only the structure but also the functioning of a cell the question arises as to what membrane changes occur during development and what relevance these have to the physiological changes and the development of the ability to withstand desiccation shown by the developing seed. However, before looking at this question it would be appropriate to describe some of the essential features of basic membrane structure. Membranes consist mainly of lipids - the most ubiquitous being phospholipids (Benson, 1964; Simon, 1974) and proteins. It is generally accepted that the integrity of the membrane originates from the amphipathic properties of the phospholipids and the ability of the phospholipids to participate in both lipid to lipid and lipid to protein interactions (Van Deenan, 1966; Van Deenan et al. 1972; Simon 1974). The exact nature of the forces involved is widely debated but a generally accepted model of membrane structure based on the bimolecular lipid leaflet model made popular by Danielli and Dawson (1934) has emerged. In the current model Singer (1971) and Singer and Nicolson (1972) have postulated that the lipid bilayer forms a viscous matrix in which

proteins are embedded, some extending throughout the membrane, others being restricted to certain parts of the membrane (Fig.1A).

In the hydrated cell, membranes form semi-permeable barriers to solute movement due to the arrangement of the proteins and lipids within the membrane, however, membrane permeability can be altered. Membrane permeability can be influenced in two ways - by physical changes or by chemical changes in membrane structure. Physical changes to membranes can be brought about by such actions as the removal of water from phospholipid molecules or by temperature changes. In doing so, the actual configuration of the membrane system changes. This has been demonstrated by Luzzati and Husson (1962) and reviewed by Simon (1974), who suggest that under normal circumstances membranes exist as lamellar structures similar to the model already suggested by Singer (1971) and Singer and Nicholson (1972) (Fig.1A) with the lipid bilayers being interspersed with water layers. Removal of water or changes in the temperature alter the molecular configuration of the membrane, resulting in a hexagonal structure where the head groups of the lipid molecules are alined around water filled channels (Fig.1B). It is this structure which is believed to be permeable to solutes (Simon, 1974). Chemical changes will also influence the permeability of membranes in that loss of phospholipids or chemical modification of phospholipid structure may alter the whole pattern of bonding of the molecules involved (Van Deenan et al, 1972; Simon, 1974).

There is some evidence for changes in membrane permeability with dehydration in seeds. Simon and Raja Harun (1972) have found that initially the leakage of electrolytes from desiccated pea seeds was high but that from the first minute of immersion in

A



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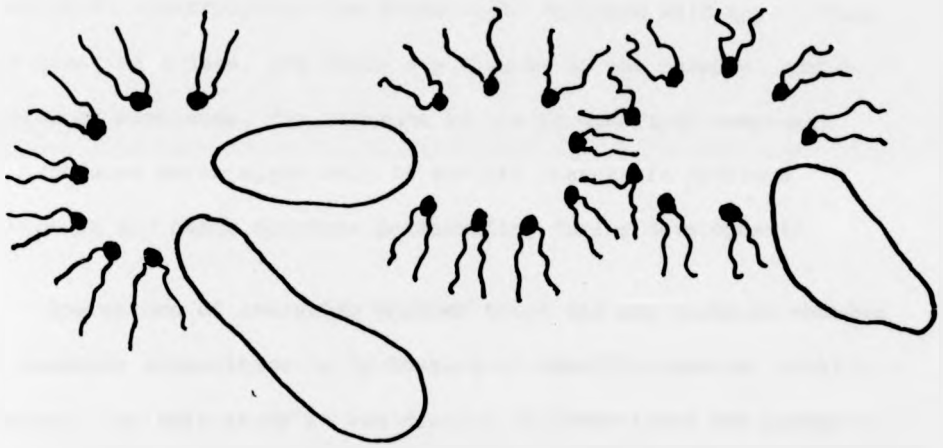

FIG. 1: The orientation of phospholipids ( ) and proteins ( ) in membrane structure.

A - the lamellar configuration with polar headgroups ( ) facing the aqueous phase on either side of the bilayer.

B - the hexagonal configuration adopted at water levels below 20%. The polar headgroups now line a series of narrow water-filled cylinders running through a relatively dry matrix (Simon, in press).

water the level of leakage declined rapidly. They interpreted this as being indicative of physical changes in membrane structure due to rehydration. Further, Buttrose and Swift (1975) have shown that in the dry pea seed the membrane system has lost its integrity but that on hydration even within eight seconds (Buttrose, 1973) membrane integrity is re-established. This, in itself would have drastic effects on the permeability properties of the membranes in the dehydrated and hydrated states. Although evidence for physical changes in membranes is accumulating there is very little known about what chemical changes may occur during development. Changes in membrane permeability during development have been suggested by the work of Matthews (1973a, b) who showed that the leakage of electrolytes from dried seeds declined with age. Thus the question arises, are there any changes in the chemical composition of membranes, for instance in the phospholipid component of membranes which might help to explain changes in membrane structure and hence membrane permeability during development?

One method of assessing whether there are any chemical changes in membrane composition is by looking at specific chemical constituents. In this study it was decided to investigate the phospholipid component of the seed partly because the role that phospholipids play in membrane structure and function is relatively more clearly understood than the role of proteins. Phospholipids consist of a diglyceride made up of an esterified glycerol with two long chain fatty acids and an alcohol moiety that gives the molecule its specific name on the third carbon position (Christie, 1973; Simon, 1974). The most common phospholipids found in plant tissues are phosphatidyl choline (PC), enthanolamine (PE), inositol (PI) and glycerol (PG) (Roughan and Batt, 1969; Kates, 1970; Donaldson

Tolbert and Schnarrenberger, 1972) with PG mainly being found in the chloroplasts and PC, PE, and PI being mainly found in the mitochondria and plasma membranes (Donaldson et al., 1972; Kates and Marshall, 1975). There are various methods of characterising the chemical composition of the phospholipid component of membranes, one of which is by using the phosphatidyl or alcohol moiety of the molecule. Examination of changes taking place during development may well help to achieve a clearer understanding of what changes are necessary before the seed can withstand drying to very low moisture levels. However it must always be remembered that membranes act as dynamic rather than static structures with the composition at any one time being the result of the balance between anabolic and catabolic processes.

One of the trends found in the evolution of the seed habit in the Angiosperms has been the evolution of increasing protection of the embryo. One aspect of this has been the evolution of a protective covering around the embryo. This protective covering may be derived from different structures such as the integuments or a combination of the integuments and the pericarp. In the pea seed it is derived from the integuments and is called the testa. Thus, the testa has an important protective function, it provides a barrier to the entry of fungal hyphae thus reducing possible loss of viability due to infection (Christensen, 1972), it provides a barrier to water movement (Denny, 1917) thus preventing premature dehydration of the embryo during development and further, its action as a barrier to water movement also prevents a too rapid influx of water into the embryo's cells during imbibition (Powell, 1977).

An additional function of the testa is suggested by work done on soybeans by Ohmura and Howell (1962). They have shown that, in developing seeds removal of the testa resulted in an increase in O_2 uptake of the older embryos, suggesting that the testa acts as a barrier to O_2 diffusion. Experimental evidence of this has been provided in peas where it has been shown that the testa acts as a barrier to O_2 movement (Wager, 1974). Such a property might inhibit the premature germination of the seeds within the pod by preventing adequate levels of O_2 reaching the embryo.

The effect of the testa on germination has been investigated by Kidd and West (1920) and Eewens and Schwabe (1975). Kidd and West (1920) found that removal of the testa from both P. sativum and Brassica alba resulted in a 100% increase in the ability of seeds to germinate but that as full maturity was approached the effect of the testa became less pronounced. Eewens and Schwabe (1975), on the other hand, concluded that the testa did not significantly increase the germination capacity of the seeds. These apparently contradictory results may be explained by Eewens and Schwabe having taken Kotowski's "co-efficient of velocity of germination" as a measure of germination capacity. Unfortunately, this parameter in effect only measures the germination rate of viable seeds, it takes no account of non-viable seeds. What Eewens and Schwabe found was that removal of the testa did not affect the rate of germination of viable seeds. Thus the effect of the testa on the germination capacity of developing seeds is still unresolved.

During this Introduction, while trying to put physiological changes in developing seeds into context, several questions have

been raised. The development of the ability to withstand desiccation (so important to the Angiosperm seed) is preceded by a fall in respiratory activity (Matthews, 1973a) but little is known about how this fall is related to other changes that are also taking place in the developing seed. The state of physiological activity as measured by O_2 uptake appears to be an important feature of the development of the ability to withstand desiccation but there are many respiratory pathways found in plants in general and in seeds in particular. Therefore, are there different respiratory pathways in use in the seed during development which do not show a fall in activity?

The ability of mature seeds to withstand desiccation is the result of an interrelated series of events occurring during development. One of the types of changes which occur are those on an ultrastructural level. These can fundamentally be regarded as changes in the membrane systems of developing seeds. Is it possible that there are specific changes in the membrane systems which can be attributed to the development of the ability to withstand desiccation?

Successful reproductive strategies not only depend on the provision of adequate supplies of reserves to ensure seedling establishment under favourable conditions but they must also provide safeguards against the precocious germination of seeds. In this context, the question arises as to whether the testa has any function other than a purely protective one and can the mobilisation of reserves in any way affect the establishment of vigorous seedlings?

MATERIALS AND METHODS

Seed Production

Seeds were produced both in glasshouses and growth rooms. Two plants per pot were established in 152mm diameter pots containing John Innes No.2 compost and grown either in a heated glasshouse (20°C ± 5°C) under a 16h photoperiod with natural light supplemented by fluorescent lights when required or in growth rooms (20°C ± 2°C) under a 16h photoperiod, light being supplied by fluorescent lights. All plants used were tagged on the day of full bloom at the first flowering node, the time of anthesis (Linck, 1961) and growth above the second flowering node was stopped by pinching off the apical meristem. Seed were removed from the first flowering node at suitable intervals during development.

Weight Determinations

The fresh weight of five replicates of five seeds was measured in all harvest series. The seeds were then dried for 48h at 105°C then re-weighed to give a measure of the dry weight. The difference between the fresh and dry weights was taken as a measure of the moisture content of the seed which was expressed throughout as mg water per seed.

Respiration Measurements

The rate of oxygen (O_2) uptake, which was used as the main measure of repiration rate, was measured with a Gilson Differential Respirometer at 20°C using either five replicates of a sample size of 20 seeds in 100 ml reaction flasks or five replicates of five seeds in 17 ml reaction flasks. One empty flask, a thermoblank

was included each time (Carver and Gloyne, 1971). Carbon Dioxide (CO_2) was removed by 10% potassium hydroxide held in the centre well, humidity was maintained by filling the side arm with water in all cases and samples were left 30 minutes to equilibrate before readings were taken.

In some experiments estimates of CO_2 output were also made by measuring the gas displacement produced by seeds respiring in flasks which had no potassium hydroxide in the centre well then measuring the O_2 uptake as before. CO_2 output was calculated by adding the increase in gas in the flasks without potassium hydroxide to the volume of O_2 taken in. Respiration rate, whether measured as O_2 uptake or CO_2 output was expressed as µl gas $seed^{-1}$ h^{-1} the figure being corrected to s.t.p.

Respiration rate was modified in different harvest series either by supplying extra respiratory substrate to the seeds in the form of 1% sucrose using water as a control or by using the respiratory inhibitors 1mM salicyclhydroxamic acid (SHAM) obtained from Sigma or 1mM potassium cyanide (KCN) obtained from B.D.H. Ltd.. In all cases the solutions were supplied to the seeds (five replicates of a sample size of five seeds in 17 ml reaction flasks) which had their testas removed, by bathing the embryos in 1 ml of the appropriate solution. The embryos were never completely covered by the solutions being used.

Storage Treatments

The physiological state of freshly harvested seeds was manipulated by holding seed under different conditions before measurements were made. Six different treatments were used. In the first

seeds were placed in muslin bags and stored at approximately 100% rh at 20°C in desiccators lined with wet paper towelling and containing water in the centre well (Solomon, 1951). This maintained the original water content of the seeds at a high level. In the second and third treatments seeds were similarly placed in desiccators containing 40% or 50% rh at 20°C obtained by placing solutions of potassium hydroxide in the centre well (40% rh using 37.5g potassium hydroxide per 100g solution and for 50% rh using 33.33g potassium hydroxide per 100g solution - Solomon, 1951). The fourth, fifth and sixth treatments dried seeds at different rates. In the fourth treatment relatively slow drying of seeds was obtained in desiccators containing calcium chloride ($CaCl_2$) (drying at a rate of approximately 70% of their original water content in three days) while in the fifth and sixth treatments more rapid drying was obtained by keeping seeds on the open bench or by placing them in a ventilated incubator at 20°C. After being subjected to either one or a number of these treatments, in any one harvest series routine measurements such as weight determinations, respiration rate, carbohydrate content or seed quality were obtained.

Carbohydrate Content

Two measurements of carbohydrate content were made, ethanol soluble sugars and starch. Freshly harvested seeds were dried in a ventilated oven (80°C), ground to a fine powder, the sugars were extracted four times in hot ethanol, the ethanol evaporated off and the sugars re-suspended in 20 ml water. The starch obtained from the residue was hydrolysed with 52% perchloric acid and precipitated with potassium iodide following the method of Hassid and Neufeld (1964). The carbohydrate content of both the

ethanol soluble fraction and the solute following acid hydrolysis of the starch was then estimated as glucose equivalents using a modified Anthrone technique whereby 0.5 ml of a 2% solution of anthrone crystals dissolved in ethyl acetate was added to 2 ml of the solution being tested, 5 ml of 95% sulphuric acid were added and the mixture was left to stand for 10 minutes for the sugars and 7.5 minutes for starch then the absorption was measured at 620nm (Loewus, 1956) using a CE 272 Linear Readout Ultraviolet Spectrophotometer. A value for starch was obtained by multiplying the absorption figure obtained from the acid hydrolysed fraction by 0.90 (Hassid and Neufeld, 1964).

Seed Condition and Viability

Seed condition was measured using seeds which had been dried artificially under vacuum over anhydrous $CaCl_2$ for 24h. It was determined by soaking weighed single seeds in 20 ml of deionized water for 24h and measuring the electrical conductivity of the soak water using a Portland Electrical Conductivity Meter (Matthews, 1973a). Potassium ions in the leachate were measured using a SP90 Atomic Absorption Spectrophotometer and the sugars in the leachate were measured as before (Loewus, 1956). After 24h soaking the seed coats were removed and the embryos were placed in 1% tetrazolium chloride for 3h (Matthews, 1971). Tetrazolium chloride produces a red formazan in the presence of respiring tissue (Mackay, 1972) thus a positive staining of the embryo axis is indicative of germinability.

Laboratory germination tests were also carried out using seeds set to germinate at 20°C in sand with an 11% moisture content (Carver, 1972).

The 'coefficient of velocity of germination' (Kotowski, 1926) for seeds of different ages was determined from the following formula: $CVG = \Sigma n. 100/\Sigma(Dn)$ where CVG is the coefficient of velocity of germination, n is the number of seeds germinating on separate days and D is the number of each day after day zero.

Determinations of the total electrolytes, potassium ions and sugars present in water soluble extracts of desiccated seeds were used to relate the availability of solutes in the dry seed to the levels leached out into soak water. Single seeds were ground at 4°C and the resultant powder was extracted in 10 ml deionized water and centrifuged at 20,000g. This was repeated, the supernatants bulked and made up to 20 ml before measurements of conductivity, potassium ions and sugars were made as before.

Phospholipid Analysis

Testae were removed from either freshly harvested or dried seeds prior to lipid extraction. Phospholipids were extracted following Folch Lees and Stanley (1957) with the modification suggested by Christie (1973) using three replicates of three seeds in 30 volumes of chloroform : methanol (2:1 v/v) for each gramme of tissue. Fractionation of total phospholipids was achieved by one dimensional thin-layer chromatography (Merck silica gel $60F^{254}$ on plates 20cm × 20cm with a thickness of 0.25mm). A mixture of chloroform-acetone-methanol-acetic acid-Water (10:4:2:2:1 v/v) was used as the developing solvent (Rouser, Kritchevsky and Yamamoto, 1967). Identification of individual phospholipids was carried out by exposure to iodine vapours using pure phospholipid samples obtained from Sigma as reference standards. Quantitative

estimations of phospholipids were made by spectrophotometric determination of phosphorous as worked out by Bartlett (1959). All solutions used has 2, 6-di-tert-butyl-p-cresol an antioxidant added at a level of 75mg litre^{-1} to prevent lipid oxidation during extraction (Christie, 1973). Between lipid extraction and phosphorus determination samples were stored in glass vials under nitrogen at -20°C.

RESULTS

Section 1 - The development of the ability to withstand desiccation

One of the most interesting attributes of most seeds is their ability to withstand very low moisture contents and it is with this problem that this section is concerned. However, before any changes in the developing seed can be related to the development of this ability to withstand desiccation the normal sequence of events during development must be established. Once this is done, it may be possible to manipulate any given event in order to assess its relationship with the development of the ability to withstand desiccation by the seed. In order to clarify this further this section has been divided into five sub-sections - changes during normal development; changes in seeds held in humid storage; a practical use for the humidity storage of pea seeds; the effect of sucrose on seed respiration, and seed development in relation to the whole plant.

Changes during normal development

Some of the changes which occur during development of freshly harvested seeds were followed in two separate harvest series. In the first series (Fig.2A) changes were followed from 15 to 34 DAFB (days after full bloom) and in the second series (Fig.2B) from 16 to 36 DAFB. In both cases dry weight and starch content, when expressed on a per seed basis increased throughout the period when seeds were being harvested. Moisture content, the rate of O_2 uptake and sugar content, on the other hand, first rose and then fell. In the first series (Fig.2A) a sharp decline in respiration rate was seen after 20 days, it was accompanied by a more gradual

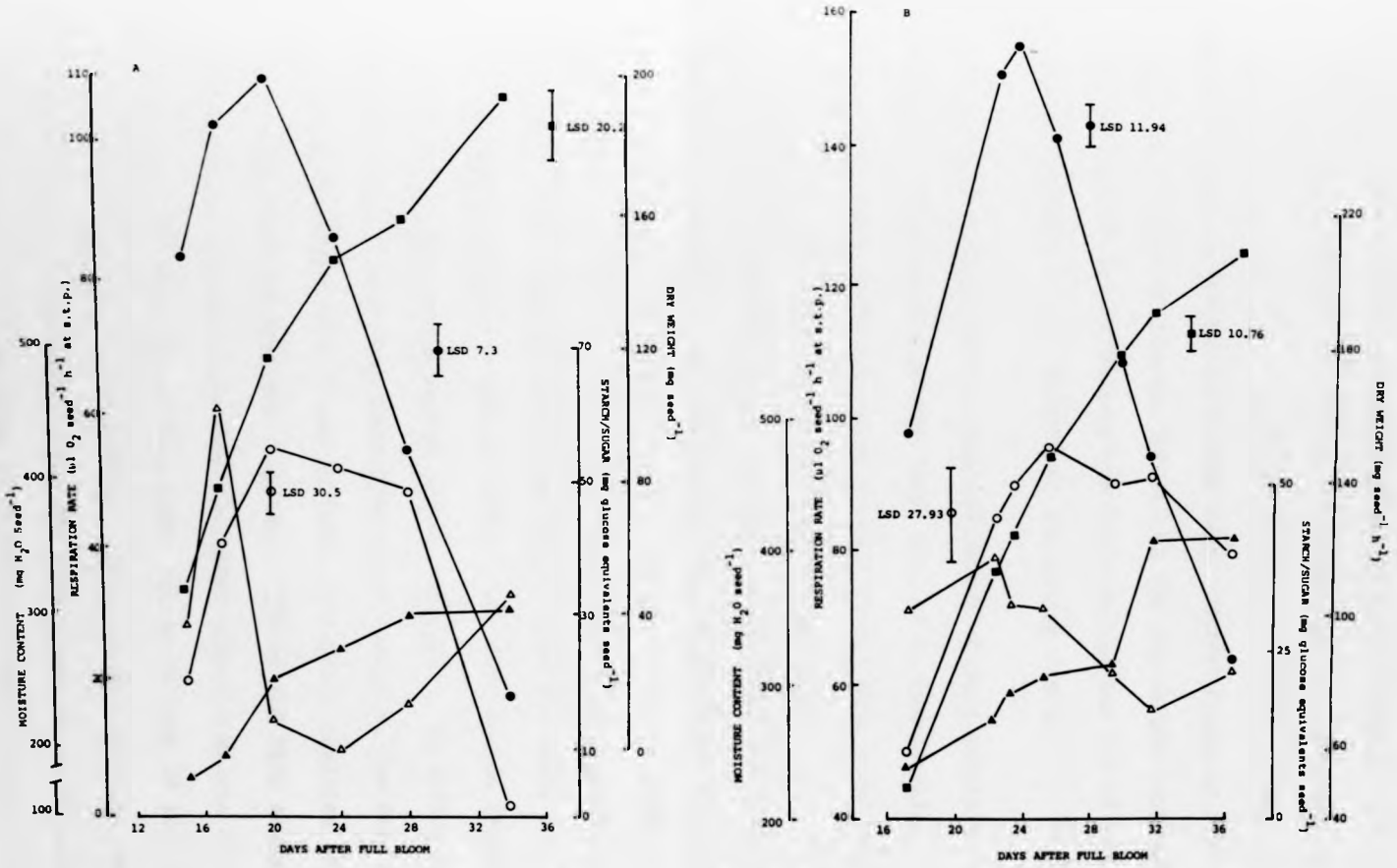

FIG.2 Changes in dry weight (■), moisture content (O) (mean of 5 replicates of 5 seeds), respiration rate (●) (mean of 5 replicates of 20 seeds), sugar (Δ) and starch (▲) levels (bulked sample of 5 seeds) in pea seeds during normal development in A harvest series 1 and B harvest series 2.

decline in moisture content and was preceded by a fall in sugar content. A similar pattern was evident in the second series (Fig.2B) with the decline in respiration rate occurring at 23 DAFB, sugar content fell by 22 DAFB but moisture content continued to increase until 26 DAFB. After 24 days in the first series and after 31 days in the second series sugar content began to rise again and appears to account for 46% of the dry weight increase seen between 28 and 34 DAFB in the first series and 34% of the increase between 21 and 36 DAFB in the second series.

In both harvest series some indication of seed condition and viability following desiccation was obtained by immediately desiccating some seeds harvested at each sample time under vacuum over $CaCl_2$. The leaching of electrolytes and sugars from desiccated seeds expressed per gramme seed dry weight declined throughout the period studied in both harvest series (Figs. 3A and 3B) with the fall in sugars being most marked between 16 and 24 DAFB in the first series (Fig.3A) and 17 to 23 DAFB in the second series (Fig.3B). The level of potassium leached from the seeds fell most markedly between 20 and 28 DAFB in the first series (Fig.3A) and 22 to 31 DAFB in the second series (Fig.3B). In series one (Fig.3A) none of the five desiccated seeds tested at the first three sample times (15, 17 and 20 DAFB) showed any staining of the embryo axis with tetrazolium chloride. Two of the five seeds tested at the fourth sample time (24 DAFB) showed complete staining of the axis and four out of five seeds tested at both 28 and 34 DAFB showed complete axis staining. This pattern was also seen in the second harvest series (Fig.3B) with three out of 15 seeds showing complete axis staining at the fifth sampling time (29 DAFB),

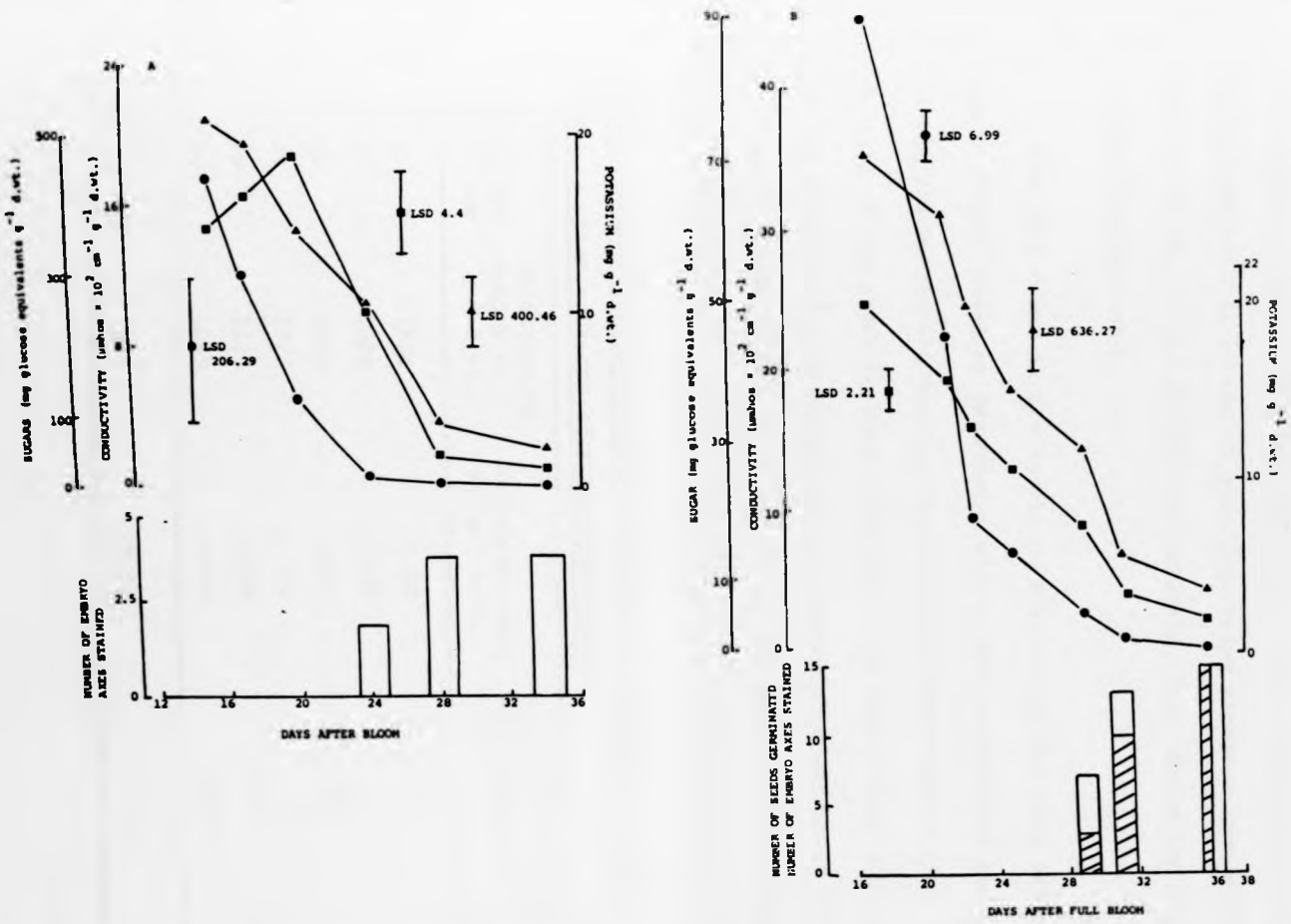

FIG.3 Changes during normal development in electrolytes (▲), potassium ions (■) and sugars (●) (mean of 5 single seeds) and in viability measured by positive staining of the embryo axis with tetrazolium chloride in A - harvest series 1 and B - harvest series 2.

10 out of 15 seeds showing complete axis staining at 31 DAFB and all 15 axes staining completely at 36 DAFB. These estimates of seed viability were further checked in the second harvest series by subjecting 15 desiccated seeds at each sampling time to germination tests in sand. The germination of normal seedlings increased throughout with 7, 13 and 15 seedlings being produced from seeds sampled at 29, 31 and 36 DAFB respectively, thus confirming the tetrazolium estimates.

The fall found in the levels of solutes leached from desiccated seeds (Fig.3) could be associated with either or both the total levels present in the seed, expressed per gramme seed or with the ability of the seeds to retain solutes. No significant differences in the total levels of potassium and electrolytes extracted from desiccated seeds of the first harvest series were detected (Table 1).

TABLE 1

Total conductivity, potassium ion and sugar (mean of 5 seeds) found in water extracts of desiccated seeds

Days after full bloom	Conductivity (μmhos cm^{-1} g^{-1} d.wt.)	Potassium ions (mg g^{-1} d.wt.)	Sugars (mg glucose equivalents g^{-1} d.wt.)
15	1924	12.58	401
17	1898	14.18	367
20	1942	16.60	165
24	1923	17.62	81
28	2177	21.54	119
34	2055	19.88	145
L.S.D. ($p < 0.05$)	307	10.54	68

The level of sugars extracted from the same seeds fell until 24 DAFB after which time they tended to increase. The low level of leaching from desiccated seeds seen in the first harvest series after 28 DAFB for both potassium and total electrolytes (Fig.3) and after 24 DAFB for sugars appears to be associated with retention rather than a fall in the solute levels found in the seed. This improved retention of solutes seen in developing seeds will be considered later in this thesis.

The ability to withstand severe desiccation exhibited by the mature seed appears to develop after a fall in physiological activity as measured by O_2 uptake. However, two other physiological changes also appear to be linked to this fall in physiological activity. Firstly, a reduction in the moisture content of the seed appears to accompany the fall in respiration rate and secondly, the level of ethanol soluble sugars appears to fall before the fall in O_2 uptake. Thus experiments were carried out in an attempt to understand the influence that both moisture and sugar levels might have on the level of physiological activity of the developing seed.

Changes in seeds held in humid storage

If a slight fall in moisture in the developing seed produced changes which resulted in a fall in the respiration rate of the seed then removal of the seeds from the parent plant when their respiration rate was rising can be used to investigate this. If the moisture content of these seeds with a rising respiration rate could be maintained then their rate of O_2 uptake would be expected to remain high. This was tested using seeds sampled at 24 DAFB

when the moisture content was increasing, and these were placed in high humidity storage (Fig.4). The respiration rate of sub-samples of five replicates of five seeds was measured at 24 DAFB and subsequently at 26 and 28 DAFB with other sub-samples of five replicates of five seeds being removed from high humidity storage at the same times and being used for weight determinations. Comparable measurements were made using seeds sampled freshly at 24, 26 and 28 DAFB (Fig.4) and these were used as a control against which the effects of high humidity storage could be compared. It was found that the moisture content of the seeds stored under high humidity was maintained under the humid storage conditions but that the respiration rate fell by 35% during the four days of humid storage (Fig.4). This was confirmed using the same techniques in a further two harvest series.

In these harvest series not only was the effect of maintaining the moisture content of the seed tested but the patterns of carbohydrate changes occurring under these humid conditions was also investigated. As before seeds were sampled in the case of the first harvest series at 23 DAFB and stored for 17 days and at 22 DAFB and stored for 13 days in the second harvest series with samples being withdrawn from humid storage at relevant intervals. Comparable measurements were again made using seeds sampled freshly at each time of sampling. For both these harvest series at each time of sampling measurements were made of weight, moisture content, respiration rate, starch and sugar changes.

For the freshly samples seeds of both harvest series (Fig.5A and Fig.5B) moisture content, starch and dry weight increased throughout the period studies. Respiration rate also increased

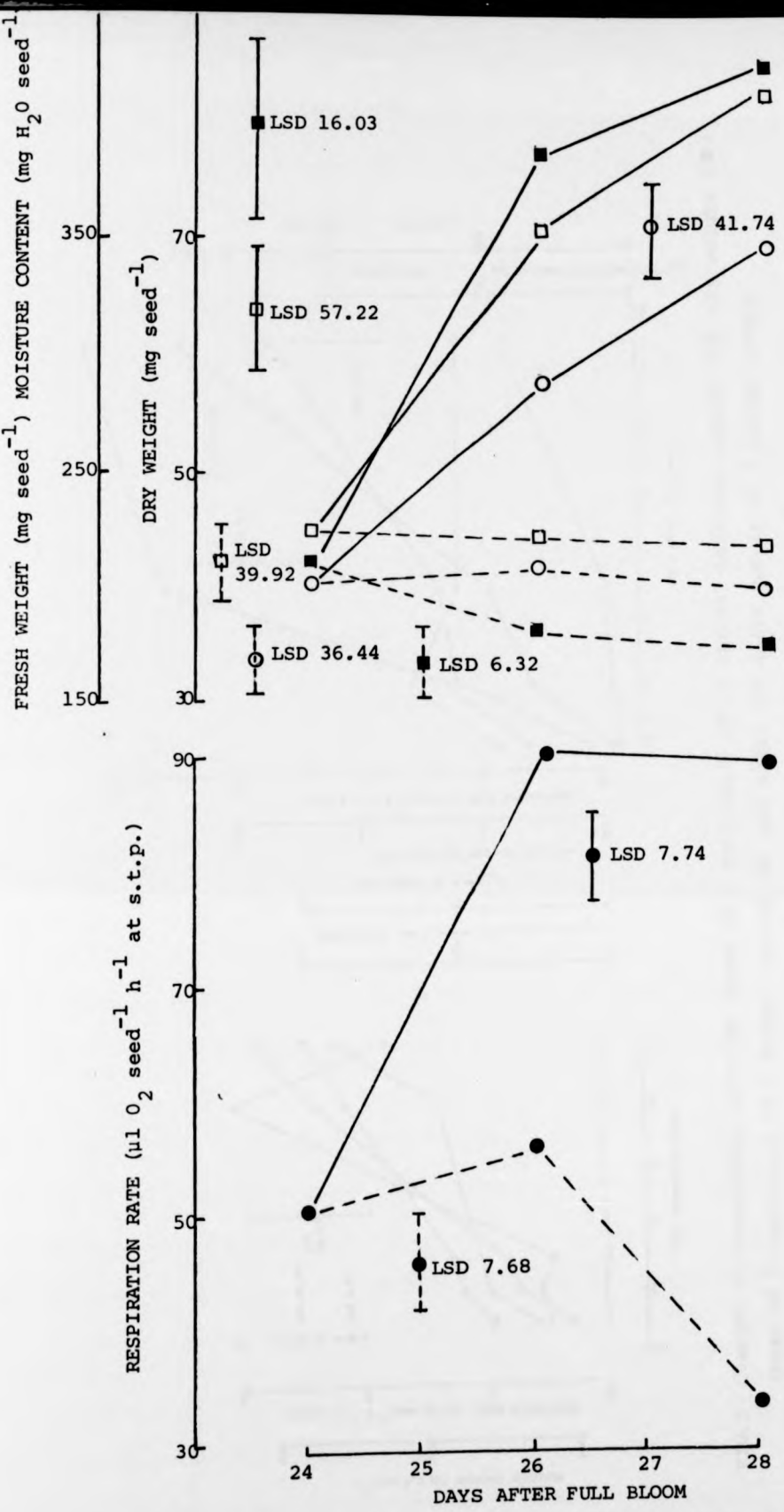

FIG.4 A comparison between the fresh weight (□), dry weight (■), moisture content (O) and respiration rate (●) of seeds sampled freshly at 24, 26 and 28 DAFB (——) and of seeds sampled at 24 DAFB then kept under 100% rh until 26 and 28 DAFB (----)

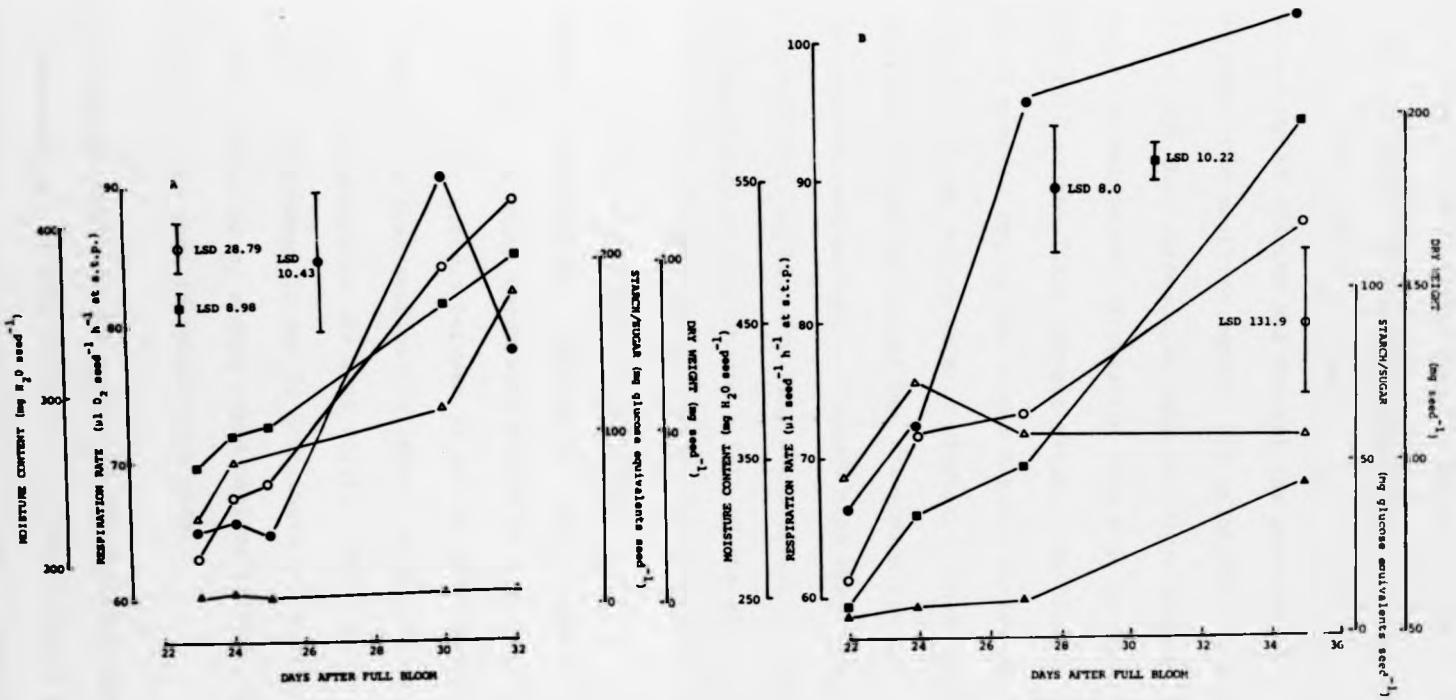

FIG.5 Changes in respiration rate (●) (mean of 5 replicates of 20 seeds), moisture content (○) dry weight (■) (mean of 5 replicates of 5 seeds), starch (▲) and sugar (△) (bulk sample of 5 seeds) levels in freshly harvested seed in A- harvest series 1 and B - harvest series 2.

throughout in the second harvest series, but started to decline by 30 DAFB in the first harvest series. Sugar content increased during the first harvest series, but remained relatively constant in the second harvest series. When the seeds stored in high humidity were sampled (Fig.6A and Fig.6B) the most striking differences were that moisture content was maintained at its original level but that respiration rate and sugar content declined. In both these harvest series respiration rate fell by about 50% while sugars fell in the first harvest series from 51.1 to 7.9mg equivalents of glucose and in the second harvest series sugars fell from 45.4 to 7.4mg equivalents of glucose. There was also a slight decline in seed dry weight but the starch levels increased slightly in both harvest series. Although both respiration rate and sugar content fell in high humidity storage, if the sugar level is of importance in influencing a fall in the respiration rate, then the sugar level would be expected to either fall before or at a faster rate than the fall in respiration rate. In the second harvest series (Fig.6B) respiration rate and sugar content fell from the time at which seeds were placed in high humidity storage but the sugar content between 22 and 24 DAFB appeared to fall at a faster rate than respiration rate. In the first harvest series (Fig.6A) the situation was less clear. Respiration rate fell throughout but between 23 and 24 DAFB there was a slight rise in sugar content followed by a more rapid decline in sugar levels between 24 and 25 DAFB than in respiration rate.

As before seed condition and viability after desiccation under vacuum was assessed for freshly harvested and humid stored seed using electrolyte leakage and tetrazolium staining of desiccated

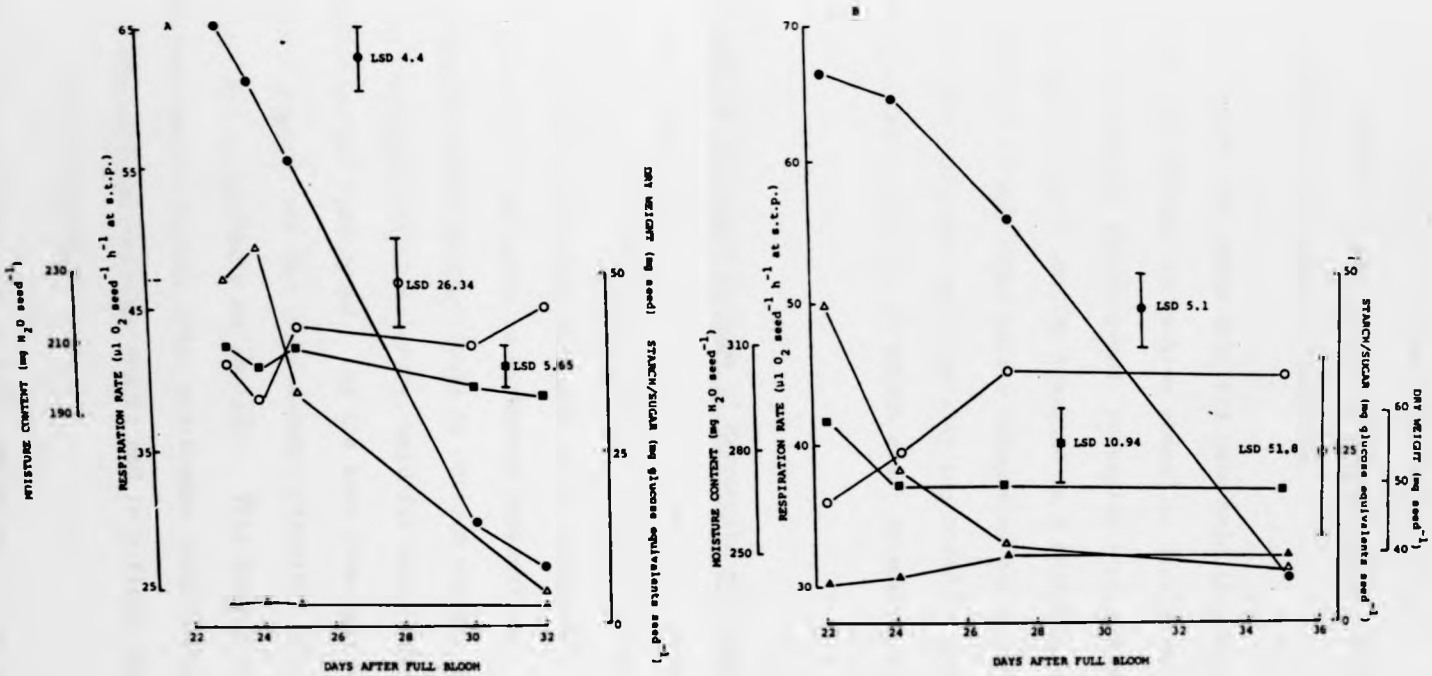

FIG.6 Changes in respiration rate (●) (mean of 5 replicates of 20 seeds), moisture content (O) dry weight (■) (mean of 5 replicates of 5 seeds), starch (▲) and sugar (△) levels in seeds harvested and stored for 0, 1, 2 and 9 days in harvest series 1(A) and for 0, 2, 5 and 13 days in harvest series 2(B) under high humidity conditions.

seeds of the second harvest series. The leakage of electrolytes, potassium ions and sugars from seeds harvested freshly before desiccation showed an initial increase then a fall (Fig.7). There was no positive tetrazolium staining of the embryo axes of the 30 seeds tested at any of the times of sampling.

Comparable values for seeds that had been held in humid storage for two days before desiccation showed no significant changes in the leakage of electrolytes, potassium ions and sugars. Another three days of humid storage resulted in a significant fall in the leachability of all three solute categories and it was only after this fall occurred that any viability indicated by positive tetrazolium staining (24 out of 30 embryo axes) became apparent (Fig.7).

A practical use for the humid storage of developing pea seeds

It has been shown that holding seeds off the plant under 'high' humidity conditions resulted in reduction in both the levels of sugars and in the respiration rate, and in the subsequent development of the ability of the seeds to withstand desiccation. It may be that a method of early harvest could be devised whereby seeds are harvested at physiological maturity, held for three days under 100% rh conditions and then dried. At the same time this would provide further support for the links between maturity, physiological activity and carbohydrate metabolism. This method early harvest has been tested firstly under glasshouse conditions using pea seeds of the cultivar Kelvedon Wonder and in a field experiment using pea seeds of the cultivar Scout.

In the glasshouse series seeds were sampled at 21, 28 and

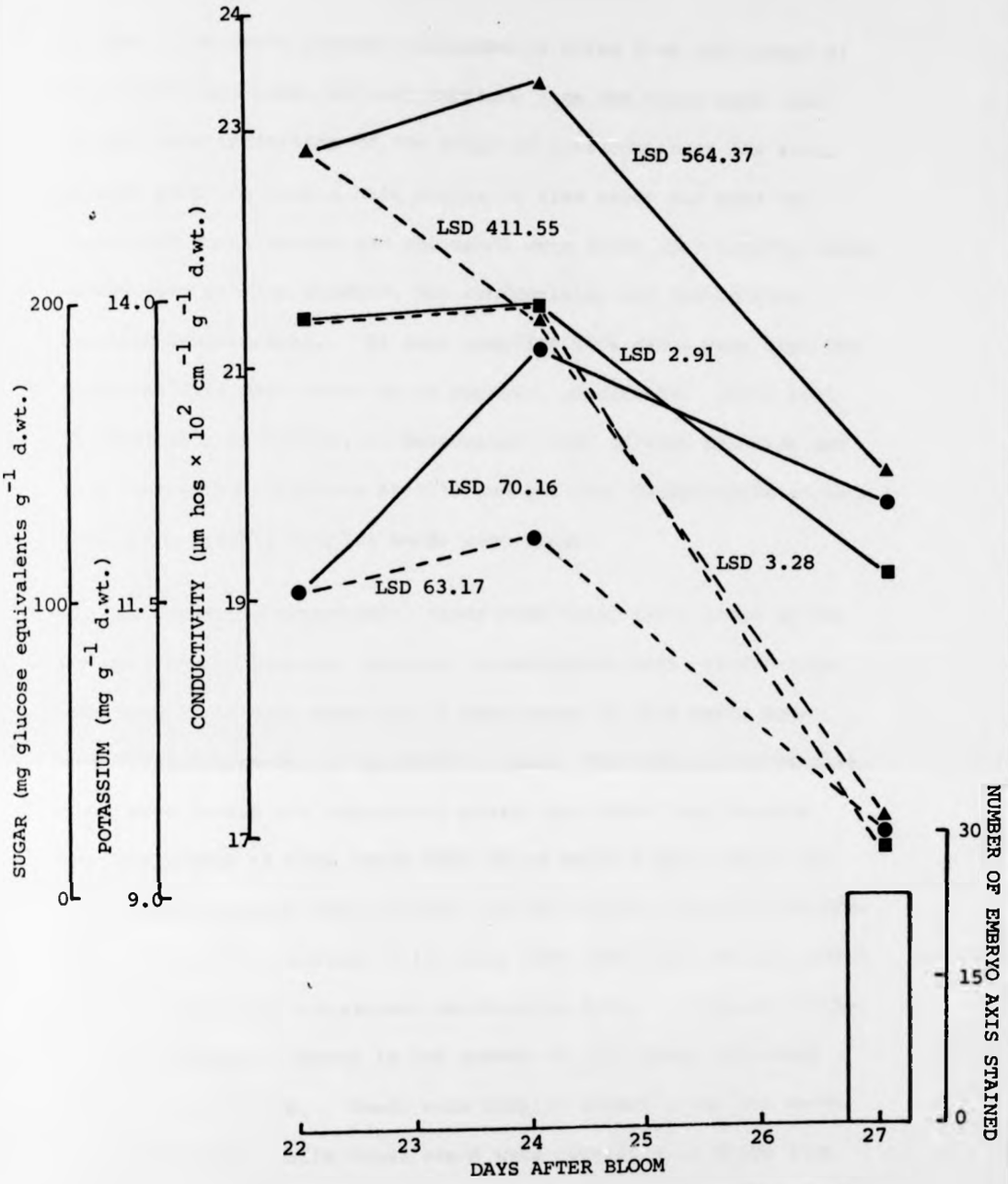

FIG.7 Changes in mean electrical conductivity (▲), potassium ions (■) and sugars (●) in a 24h leachate from 5 single seeds and in seed viability from 30 single seeds (measured by positive staining of the embryo exis with tetrazolium chloride) of freshly harvested (——) seed and seed stored (---) under 100% rh until 24 and 27 DAFB

35 DAFB. Moisture content measurements using five replicates of five seeds were taken at each sampling time and these were used to give some indication of the stage of development of the seed. At each sampling time a bulk sample of five seeds was used for starch and sugar assays and ten seeds were dried down rapidly under vacuum over calcium chloride for conductivity and tetrazolium staining measurements. At each sampling time seeds were kept for three and five days under three separate conditions: under 100% rh conditions as before, in desiccators over calcium chloride and in a ventilated incubator at 20°C and the same measurements as were made using freshly samples seeds were taken.

In the field experiment, seeds from field plots grown at the Processors' and Growers' Research Organisation near Peterborough were used. At each sampling 20 replicates of five seeds were used for measurements of moisture content, four replicates of five seeds were frozen for subsequent starch and sugar measurements, four replicates of five seeds were dried using a small still air drier for subsequent conductivity and tetrazolium staining measurements and four replicates of 15 seeds were also dried using a small still air drier for subsequent germination tests. Because of the very short growing season in the summer of 1976 seeds were only harvested at 30 DAFB. Seeds were sampled directly and the above measurements made while other seeds were subjected to three days storage under 100% rh conditions, 75% rh conditions or over calcium chloride before the above measurements were made.

In the glasshouse experiment the moisture content of seeds held under 100% rh was maintained at a high level (Table 2). Seeds held in the incubator dried more rapidly than seeds held over

TABLE 2

The moisture content (mg H_2O $seed^{-1}$; mean of five replicates of five seeds) of seeds held in 100% rh, over calcium chloride and in an incubator at 20°C for 0, 3 and 5 days following normal sampling at 21, 28 and 35 DAFB

DAFB	Days Stored	100% rh	Calcium Chloride	Incubator
21	0	318b	318b	318b
	3	348ba	59c	20c
	5	332b	14c	14c
28	0	396a	296a	396a
	3	376a	70c	24c
	5	364a	22c	19c
35	0	333b	333b	333ab
	3	311b	69c	33c
	5	299b	27c	18c

The same letters in each column are not significantly different according to Duncan's Multiple Range Test $p \leq 0.05$

calcium chloride. The moisture content peaked between 28 and 35 DAFB and the peak in moisture content occurs at approximately the same time as full physiological maturity.

When the carbohydrate content of the seeds was examined it was seen that the starch content increases throughout development (Table 3). In the very young seeds at 21DAFB starch tends to increase in seeds kept under 100% rh while in the 21 DAFB seeds kept over calcium chloride or in the incubator the starch levels remain relatively constant. This is also true of the 28 DAFB seeds kept under all conditions but the starch levels tend to decrease in the 35 DAFB seeds kept under all conditions. The sugar content of the seeds stored under high humidity conditions

showed a marked decrease in sugar content at all sampling times. The sugar content of seeds stored under drier conditions, i.e. over calcium chloride or in the incubator showed a much more variable pattern of sugar content with a general tendency for the sugar levels to remain constant (Table 3). Thus the treatments used, achieved both different rates of drying of the seed and different sugar levels which have been shown to alter the physiological state of the seed.

TABLE 3

The starch and sugar levels (mg glucose equivalents seed^{-1}; bulk sample of five seeds) of seeds held for 0, 3 and 5 days under 100% rh, over calcium chloride and in an incubator at 20°C following sampling at 21, 28 and 35 DAFB

DAFB	Days Stored	100% rh		Calcium Chloride		Incubator	
		Starch	Sugar	Starch	Sugar	Starch	Sugar
21	0	9.9	52.3	9.9	52.3	9.9	52.3
	3	-	11.8	7.2	29.0	14.2	50.9
	5	15.9	7.2	9.5	47.7	9.6	40.9
28	0	20.5	10.9	20.5	10.9	20.5	10.9
	3	20.5	5.7	24.0	12.6	26.4	16.9
	5	19.5	3.7	18.6	18.1	26.5	15.3
35	0	45.6	21.4	45.6	21.4	45.6	21.4
	3	23.7	15.8	24.0	17.8	24.4	17.3
	5	19.4	8.6	29.2	16.2	31.3	14.7

When the seed condition after desiccation was examined the differences between the treatments used was more evident. In the youngest seed the 24h conductivity of the leachate from dried

seeds remains high although it did decrease in the high humidity stored seed but there were no viable seeds indicated by positive staining of the embryo axes found (Table 4). At 28 DAFB when there was a peak in moisture content (Table 2) and a decline in sugar content (Table 3) which would indicate the beginning of a decline in physiological activity, the embryo condition improved more markedly in seeds kept under high humidity conditions indicated by both a more rapid decline in conductivity and a more rapid increase in viability when compared to seeds dried more rapidly i.e. over calcium chloride or in the incubator (Table 4). Seeds dried most rapidly in the incubator had the highest conductivity and the lowest viability (Table 4). In the oldest seed at 35 DAFB the conductivity in all treatments had been reduced to a very low level and all the seeds tested were viable (Table 4). This suggests that it is possible to harvest early and still produce viable seeds providing that the physiological activity of the seeds has begun to fall. In this experiment, this was indicated by a peak in moisture content and a fall in sugar content.

In the field experiment seeds were sampled at 30 DAFB which was approximately the time at which the peak in physiological activity occurred. In this harvest series measurements of moisture content, starch and sugar content were made prior to desiccation and after seeds were desiccated measurements of embryo condition, i.e. conductivity of a 24h leachate and tetrazolium staining of the embryo axes; and germination were made. Seeds were also sampled three days after being subjected to the different treatments used. The high humidity conditions provided by 100% and 75% rh maintained the moisture content of the seed at a high level

TABLE 4

Seed condition measured as the electrical conductivity of seed leachate (μmho cm^{-1} g^{-1} d.wt.; means of five single seeds) and tetrazolium staining of the embryo axes (number out of five) after desiccation and holding for 0, 3 and 5 days under 100% rh, over calcium chloride or in an incubator at 20°C

DAFB	Days Stored	100% rh		Calcium Chloride		Incubator	
		conductivity	viability	conductivity	viability	conductivity	viability
21	0	3344a	0	3344b	0	3344a	0
	3	2967ab	0	4013a	0	3805a	0
	5	2473b	0	4240a	0	3456a	0
28	0	1599c	2	1599c	2	1599b	2
	3	896d	5	1626c	2	1532b	0
	5	805d	5	1082cd	5	1478b	3
35	0	654d	5	654d	5	654c	5
	3	683d	5	780d	5	845cb	5
	5	604d	5	684d	5	961cb	5

The same letters in each column are not significantly different according to Duncan's Multiple Range Test $p \leq 0.05$

(Table 5) while the sugar content fell by approximately 60%. The starch content remained high under the 75% rh conditions but was reduced under the 100% rh conditions. The seeds which were held over calcium chloride showed a reduction in moisture content with no corresponding fall in sugar content although the starch content was lower (Table 5). Thus the higher humidity conditions again produced a reduction in physiological activity as a result of sugar stress which the drying treatment did not produce.

TABLE 5

The moisture content (mean of 20 replicates of 5 seeds) and the sugar contents (4 replicate of 5 seeds) of field grown seed peas at 30DAFB and after being subject to either 3 days at 100% rh, 75% rh or over calcium chloride

Treatment	Moisture Content (mg H_2O $seed^{-1}$)	Starch (mg glucose equivalents $seed^{-1}$)	Sugar
Initial	266.23a	29.92a	17.52a
3 days 100% rh	228.70a	14.23b	6.48b
3 days 75% rh	135.93b	27.21a	6.97b
3 days $CaCl_2$	2.92c	14.83b	17.55a

The same letters in each column are not significantly different according to Duncan's Multiple Range Test $p \leq 0.05$

When the embryo condition of seeds subjected to the different treatments was examined it was seen (Table 6) that seeds desiccated immediately after harvest had a high conductivity coupled to a low viability measured by the tetrazolium staining of embryo axes. Seeds held under drier conditions, i.e. over calcium chloride, prior to desiccation had a lower conductivity than seeds dried directly but this was still high and again there was a low viability (Table 6). The higher humidity treatments results in seeds after

desiccation which showed lower conductivities (100% rh producing the lowest conductivity) and higher seed viability. When desiccated seed after each treatment were set to germinate in sand those dried directly and those dried after being held over calcium chloride had a percentage germination of 0 and 13.3% respectively. Those seeds that had been subjected to high humidity storage i.e. 100% and 75% rh had percentage germinations of 80%. Thus, on the scale that this field experiment was carried not only is the idea of holding seeds that had reached a peak of physiological activity, under high humidity conditions in order to prematurely induce the development of the ability to withstand desiccation feasible; but the influence of carbohydrate metabolism on physiological activity and hence the ability of seeds to withstand desiccation is further demonstrated.

TABLE 6

The effect of post-harvest treatment on the seed condition of field grown seed peas. Conductivity readings are means of 20 single seeds, tetrazolium staining of axes are numbers out of 20 and % germinations are each of 60 seeds

Treatment	Conductivity (μmhos g^{-1} d.wt.)	Embryo Axis Staining (% positive)	% Germination
Direct Dried	2123.35a	15b	0c
3 days $CaCl_2$	1949.84a	20b	13.33b
75% rh	1264.64b	85a	80a
100% rh	700.43c	90a	80a

The same letters in each column are not significantly different according to Duncan's Multiple Range Test. The arc sin transformation has been used for percentages. $p \leq 0.05$

The effect of sucrose on seed respiration

Under high humidity conditions the moisture content of harvested seeds was maintained at a high level but a significant decrease in respiration rate still occurred. Furthermore, in the second harvest series involving high humidity storage and in both the harvest series measuring normal changes during development the fall in respiration rate was preceded by a fall in ethanol soluble sugars. It was only after these changes had occurred in the seeds stored under high humidity conditions that an improvement in their ability to withstand desiccation, as indicated by retention of solutes and positive tetrazolium staining became apparent. Thus, if the fall in respiration rate found in seeds kept in humid storage resulted from a fall in carbohydrate levels then addition of extra carbohydrates, in this case sucrose was used, to these seeds with a declining respiration rate would be expected to produce an increase in respiration rate. Such an increase would not be expected to occur in freshly harvested seeds. These predictions were tested using three separate harvest series, comparisons being made between seeds stored under 100% rh and seeds harvested freshly from the plant.

In all three harvest series, seeds were harvested when the moisture content and more importantly, the sugar content of the seeds were still increasing (Table 7). In series 1, seeds were harvested at 23 DAFB and kept in 100% rh for nine days. In series 2 seeds were harvested at 22 DAFB and stored for 13 days while in series 3 seeds were harvested at 17 and 21 DAFB and stored for six days. Seeds were freshly harvested 32 DAFB in seriesl, 35 DAFB in series 2 and 17 and 21 DAFB in series 3 (Table 7).

TABLE 7

Starch and Sugar levels (mg glucose equivalents seed^{-1}) of seeds sampled freshly and seeds stored under 100% rh at the beginning and end of three separate harvest series. Values were obtained from bulked samples of five seeds at each sampling time

Carbohydrate level (mg glucose equivalent seed^{-1})	Series 1		Series 2		Series 3			
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Sugar								
Initial	48	48	45.4	49.4	7.0	7.0	27.3	27.3
Final	180.6	4.8	57.9	7.38	7.0	2.45	27.3	4.24
Starch								
Initial	3.1	3.1	5.1	5.1	2.0	2.0	5.9	5.9
Final	8.3	3.1	45.1	9.4	2.0	3.0	5.9	8.3

Initially the respiration rate of the whole seed was measured; the testa was then removed and the respiration rate measured again. The same procedure was used for both freshly harvested and stored seed. The exposed embryos were then bathed in either distilled water of 1% (w/v) sucrose for 1h after which their respiration rate was again measured, the embryos remaining in the solutions in the reaction flasks.

The respiration rate of intact seeds kept in 100% rh had decreased by about 50% in all series (Table 8). Removal of the testa resulted in little change in the rate of O_2 uptake of the embryo. The addition of water to the embryos likewise produced no significant change in the respiration rate but the addition of sucrose produced a significant increase in respiration rate in all series. In series three the effect was more pronounced in the older seeds.

TABLE 8

The influence of testa removal and the addition of water or 1% sucrose on the respiration rate (µl O_2 $seed^{-1}$ h^{-1} at s.t.p.) of seeds stored under high humidity conditions in three harvest series (means of five replicates of five seeds)

Treatment	Series 1	Series 2	Series 3	
	23 + 9 days storage	22 + 13 days storage	17 + 6 days storage	21 + 6 days storage
with testa	26.8	30.9	29.3	47.1
without testa	22.9	34.7	21.7	41.8
without testa water added	24.9	43.0	21.8	44.2
without testa sucrose added	36.4	50.0	29.7	62.7
L.S.D. ($p < 0.05$)	4.72	8.96	5.97	14.38

In the freshly harvested seed (Table 9) removal of the testa resulted in an increase in the respiration rate of the exposed embryo in all series with the exception of the youngest seed (17 DAFB). This suggests that the embryos of the seeds examined had a high potential for respiration rate which the testa prevented from being realised. The respiration rate of these exposed embryos was higher than that of the embryos following humid storage. The addition of water to these freshly harvested embryos reduced respiration rate significantly in all cases (Table 9). This effect may have resulted from limited oxygen availability caused by slow O_2 diffusion through the water surrounding the embryos in the reaction flasks (James, 1953 and Ohmura and Howell, 1960). The sugar solution had a similar effect to that of water, i.e. respiration rate was reduced.

TABLE 9

The influence of testa removal and the addition of water or 1% sucrose on the respiration rate (µl O_2 $seed^{-1}$ h^{-1} at s.t.p.) of freshly harvested seed in three harvest series (mean of five replicates of five seeds)

Treatment	Series 1	Series 2	Series 3	
	32 days	35 days	17 days	21 days
with testa	65.1	101.0	53.2	85.4
without testa	131.9	154.0	31.4	97.5
without testa plus water	53.4	119.1	15.5	67.7
without testa plus 1% sucrose	46.4	135.6	24.0	89.9
L.S.D. ($p < 0.05$)	19.78	29.88	8.34	26.47

Thus it can be said that the development of the ability to withstand desiccation displayed by mature pea seeds is connected with the state of physiological activity of the seed. This is itself influenced by metabolic events, with the level of soluble carbohydrates apparently having a strong influence on the level of physiological activity of the seed.

Most of the sugars involved in carbohydrate metabolism of the developing seed are supplied to the seed from the parent plant. If a reduction in physiological activity is brought about by a fall in the level of sugars then it should be possible to reduce the supply of sugars from the parent plant to the seeds at a stage where the respiration rate is still rising and the development of the ability to withstand desiccation should be obtained earlier.

Seed development in relation to the whole plant

During normal development, the ability of seeds to withstand desiccation coincides with a fall in the respiration rate of the seed, which is in turn preceded by a fall in sugar content of developing seeds. This reduction in both respiration rate and sugar content has been achieved by storing seeds under 100% rh conditions and has further resulted in the early development of the ability to withstand desiccation. So far, all the work that has been carried out in this thesis has used seeds detached from the parent plant. The question arises as to whether the fall in respiration rate necessary for the development of the ability to withstand desiccation can be brought about prematurely by reducing the sugar supply to the developing seed from the parent plant.

This was initially tested by growing plants in growth rooms until they showed a rising phase in physiological activity. The lights were then turned off in one growth room so stopping photosynthesis and presumably reducing the sugar supply from the parent plant while as a control, seeds were allowed to develop normally in another growth room. In this harvest series, the moisture content and dry weight (five replicates of five seeds) continued to increase in both the seeds grown normally and in the seeds grown in the dark (Fig.8). Furthermore, the respiration rate of five replicates of five seeds taken from plants grown in the dark showed the same pattern of increase as the respiration rate of five replicates of five seeds from plants grown normally (Fig.8). The sugar content of seeds in this harvest series was not measured but the continued increase in dry weight suggested that assimilates were still being transferred to the seed despite the dark conditions.

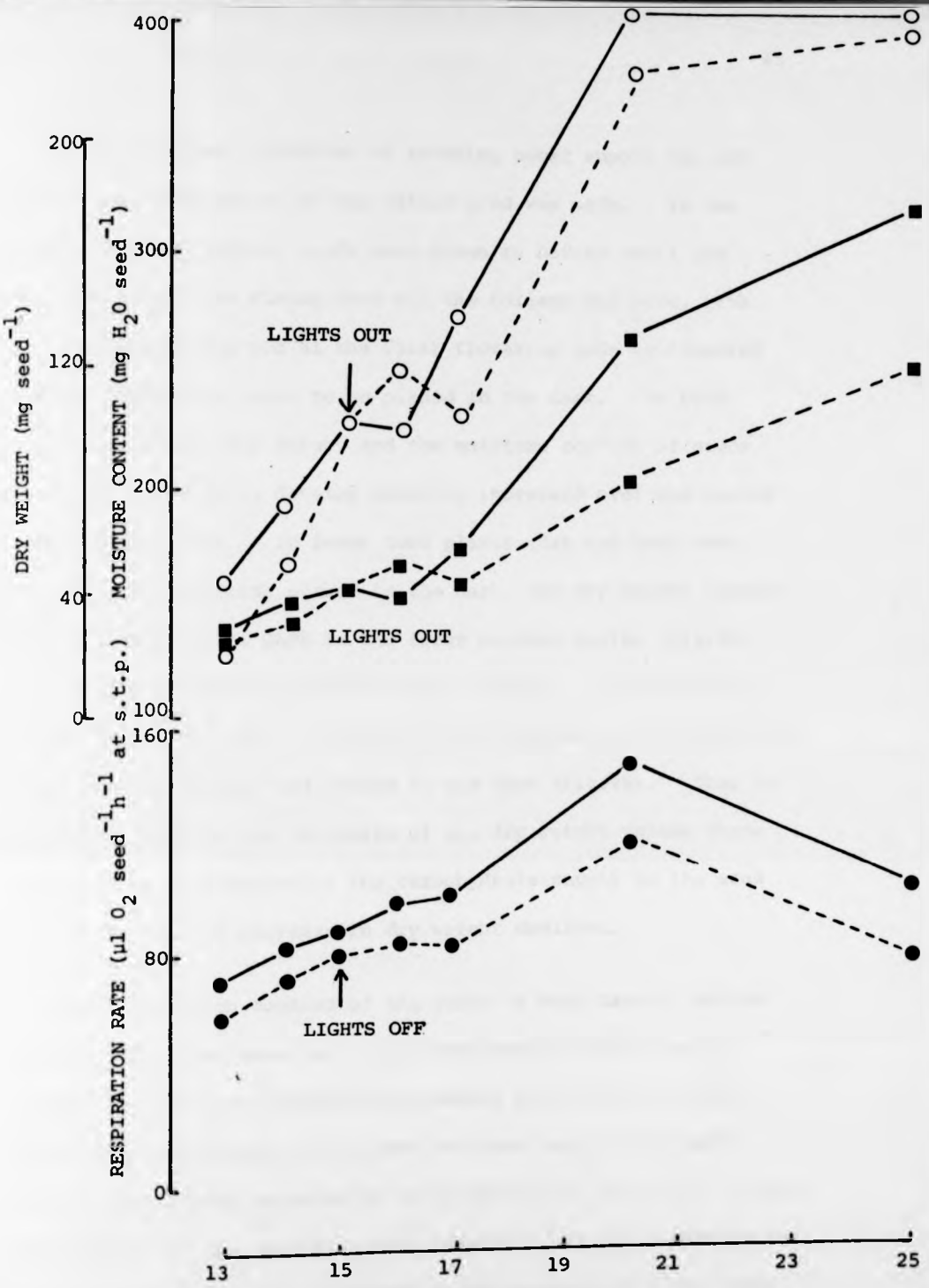

FIG.8 Respiration rate (●), moisture content (O) and dry weight (■) of seeds grown in the light (——) and of seeds initially grown in the light then transferred to the dark (---). All points are a mean of 5 replicates of 5 seeds.

As the original intention of reducing sugar supply was not achieved, a modification of the method used was made. In two separate harvest series, seeds were grown as before until the respiration rate was rising then all the foliage and pods, with the exception of the pod at the first flowering node were removed from the plants that were to be placed in the dark. In both harvest series the dry weight and the moisture content of seeds from plants allowed to develop normally increased over the period studied (Fig.9A, B). In seeds from plants that had both been defoliated before being placed in the dark, the dry weight increased between 21 and 24 DAFB in the first harvest series (Fig.9A) but the rate of increase declined after 24DAFB. In the second harvest series the rate of increase in dry weight slowed down after plants were defoliated and placed in the dark (Fig.9B). Thus in both harvest series, on the basis of the dry weight values there appears to be a reduction in the carbohydrate supply to the seed in that the rate of increase in dry weight declined.

When the starch content of the seeds in both harvest series was examined it was seen that the starch content continued to increase in both seed removed from control plants and in seeds removed from defoliated plants that had been kept in the dark (Fig.10) thus, there appeared to be no hydrolysis of starch. The sugar content of the control plants allowed to develop normally in the first harvest series fluctuated rather markedly but the sugar content of seeds from the defoliated, dark treated plants decreased markedly after the plants were defoliated and placed in the dark (Fig.10A). A similar pattern of changes was found in the second harvest series (Fig.10B), with a marked decline in sugar after

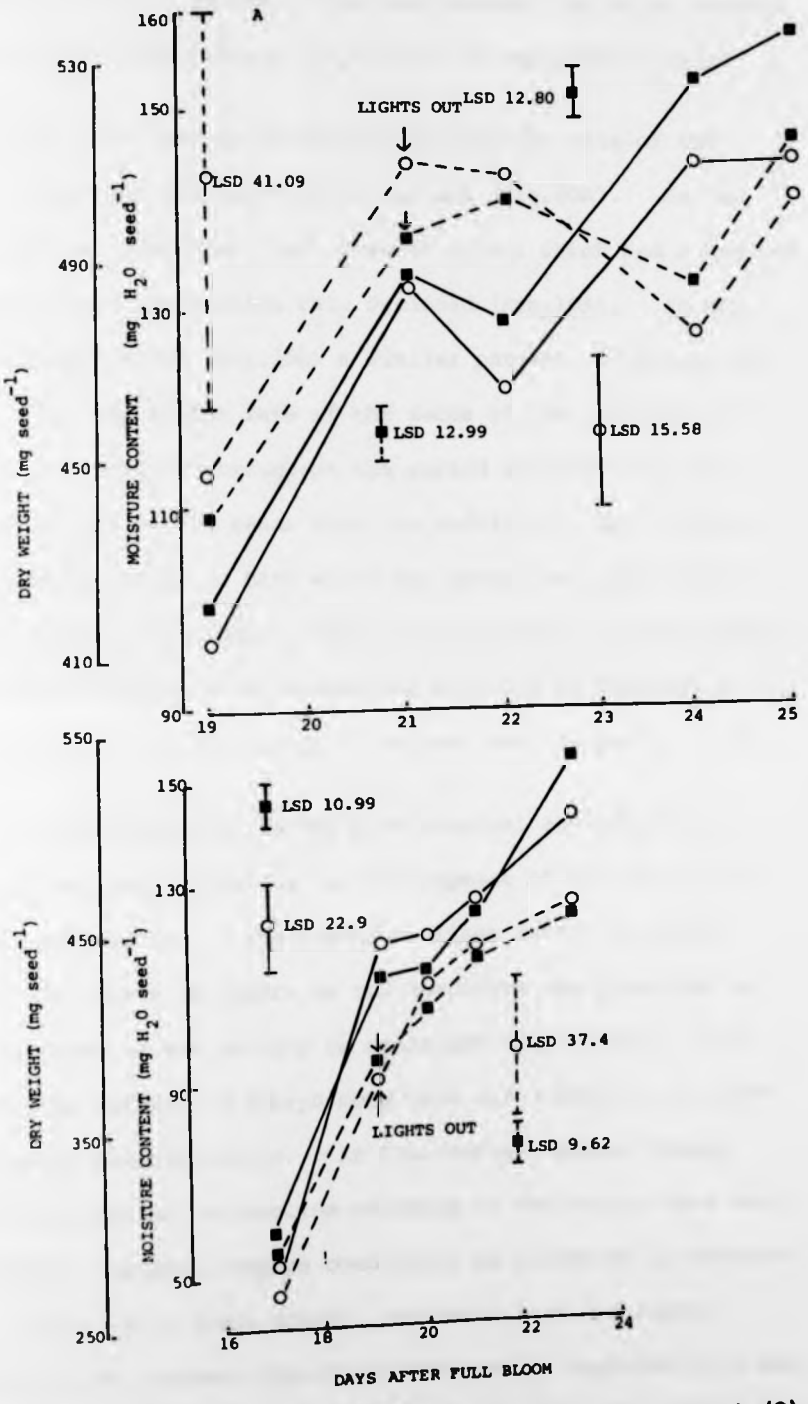

FIG.9 Changes in dry weight (□) and moisture content (O) (mean of 5 replicates of 5 seeds) in freshly harvested seeds taken from plants grown normally (——) and from plants initially grown in the light then defoliated and placed in the dark (---) in A - the first harvest series and B - the second harvest series.

plants had been defoliated and placed in the dark and little change in the sugar levels of seeds from control plants. Thus defoliation prior to placed in the dark reduced the sugar content of the seeds but what effect did it have on respiration rate?

In the first harvest series the respiration rate of the control seeds remained relatively constant (Fig.10A). In the seeds from the defoliated, dark treated plants which had a reduced sugar supply the respiration rate declined (Fig.10A). In the second harvest series (Fig.10B) a similar pattern of changes was found. The respiration rate of the seeds of the control plant continued to increase throughout the period studied while the respiration rate of the seeds from the defoliated, dark treated plants declined after 20 DAFB which was after the sugar levels began to decline (Fig.10B). Thus, it is possible to bring about a premature reduction in physiological activity of developing seeds by restricting the supply of sugars from the parent plant.

Previously reduction in the physiological activity of pea seeds has been accompanied by the development of the ability to withstand desiccation. The question arises as to the effect reducing the supply of sugars to the seed from the plant had on the development of the ability to withstand desiccation. This was tested by estimating embryo condition and viability of seeds in the second harvest series. In this harvest series viable seeds as measured by tetrazolium staining of the embryo axes were never found. However, embryo condition, as estimated by measurements of electrolyte conductivity, potassium ions and sugars present in a 24h leachate from desiccated seeds, improved with age in both the control and the defoliated dark treated seeds (Fig.11).

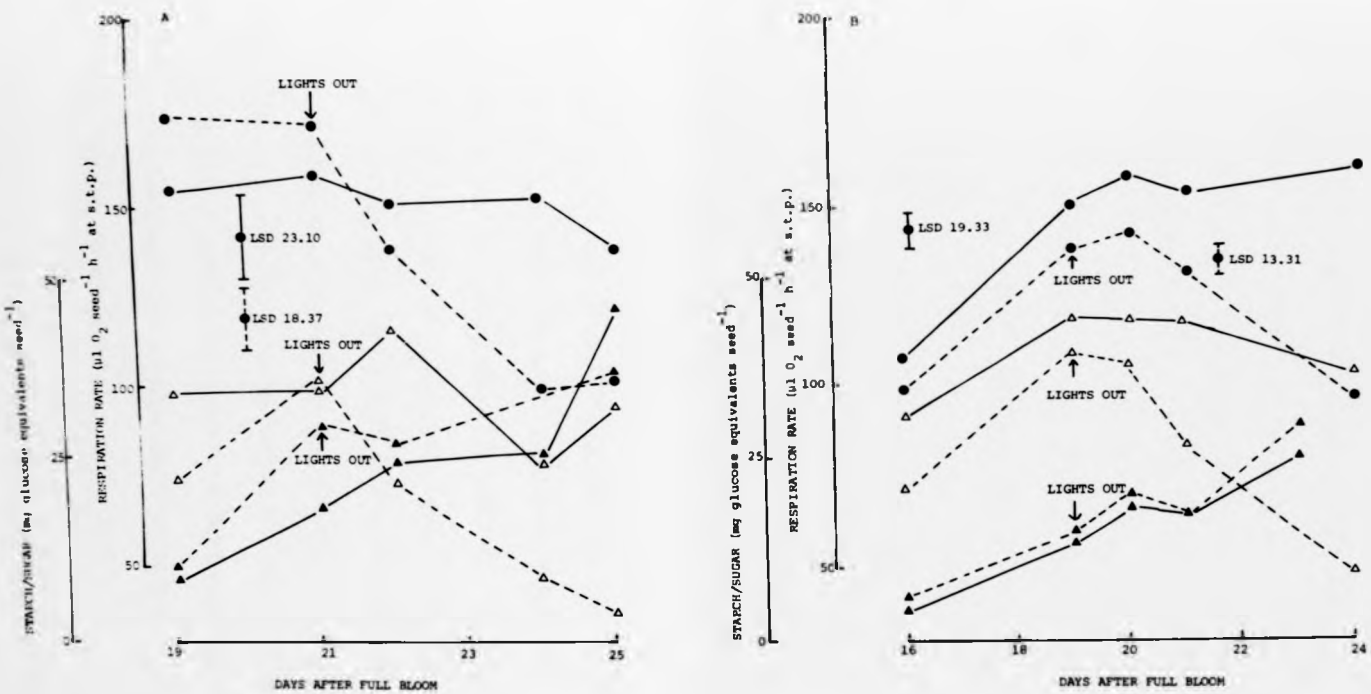

FIG.10 Changes in respiration rate (●) (mean of 5 replicates of 5 seeds) starch (▲) and sugar (Δ) levels (bulk sample of 5 seeds) found in freshly harvested seed from plants grown normally (——) and from plants initially grown in the light then defoliated and placed in the dark (---) in two harvest series A the first and B the second.

Embryo condition improved more rapidly in seeds from dark, defoliated treated plants than in seeds from control plants. This is shown by a significant decrease in conductivity between 20 and 21 DAFB in seeds from defoliated dark treated plants when compared to a significant decrease in conductivity between 21 and 22 DAFB i.e. seeds from control plants. A similar pattern of changes was found in sugar and potassium levels in the 24h leachate with a significant decline between 19 and 21 DAFB and 19 and 23 DAFB respectively in seeds from defoliated dark treated plants when compared to a significant decrease between 21 and 23 DAFB in both potassium and sugar levels in seeds from control plants (Fig.11). Therefore by reducing the supply of carbohydrates from the parent plant to the developing seed the physiological state of the seed can be reduced and the embryo condition after desiccation improved in a similar way as when high humidity storage manipulates the physiological state of the stored seed.

It is clear that the level of physiological activity of the seed is of importance in whether or not a seed can withstand drying. Physiological activity has most conveniently been measured using O_2 uptake but this may not be the best indicator to use. In the Introduction, it has been shown that there are several respiratory pathways that have been found to operate in seeds but these have been looked at mainly in germinating seeds. Little is known about whether the ability to withstand desiccation, shown by the mature seed, is related to a change in a particular respiratory pathway during development.

It can be said that the development of the ability to withstand desiccation is characterised by a reduction in the leakage of

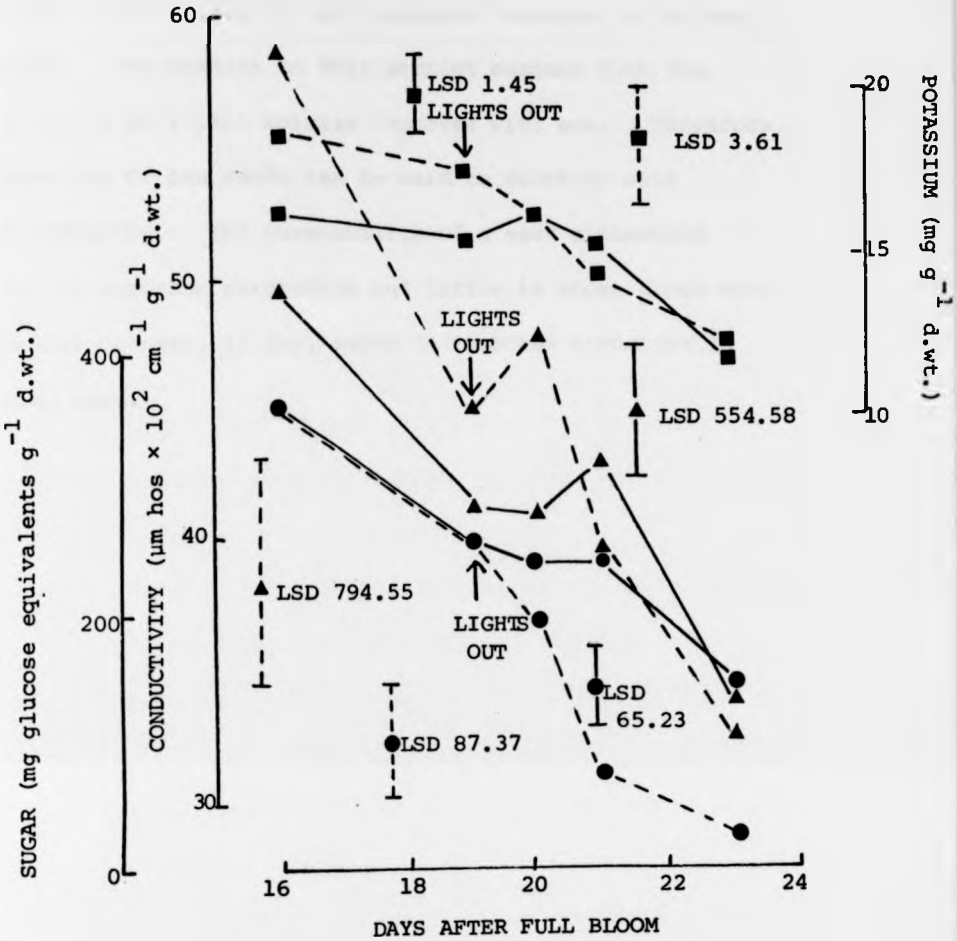

FIG.11 Comparison of conductivity (▲), potassium ion content (■) and sugar content (●) in a 24h leachate of freshly harvested seed (——) and seed stored under 100% rh (---) after being subjected to vacuum desiccation. Mean of 5 single seeds

solutes into steep water. This could be the result of either reduced solute availability or of increased retention of solutes by the seeds. The results in this section suggest that the ability of seeds to retain solutes improves with age. Therefore, the permeability of pea seeds can be said to decrease with increasing maturity. The permeability of a seed ultimately depends on its membrane structures but little is known about what basic chemical changes, if any, occur in membrane structure in a developing seed.

Section 2 : Characterisation of embryo respiration

This study has been aimed at investigating factors which might influence the ability of seeds to withstand desiccation. Measurements of factors such as carbohydrate content or physiological activity have been made using entire seeds, however, it is the embryo itself which must develop the ability to withstand desiccation. With this in mind, this section will concentrate on changes in the physiological activity of the embryo alone.

Throughout this study the main measure of physiological activity used was that of respiration rate as indicated by the rate of O_2 uptake. This type of measurement was based on the assumption that the dominant respiratory pathway in use was through glycolysis, the TCA cycle and a terminal cytochrome oxidase system. However, there may be other respiratory pathways operating during development in pea seeds. If this is so, then O_2 uptake may not be the only measurement of physiological activity that should be made. Other respiratory pathways have been found to operate in germinating pea seeds but little is known about the situation in developing seeds. Some attempt was therefore made to find out whether pathways, other than the TCA cycle were to be found during the course of development of pea seeds. This was done by measuring both O_2 uptake and CO_2 output of freshly sampled embryos with or without selective respiratory inhibitors being present in both the light and the dark.

In an experiment covering the period from 15 to 32 DAFB both the O_2 uptake and the CO_2 output of seeds (five replicates of five embryos), freshly harvested at each sampling time was measured after the testae had been removed. Both the O_2 uptake (Fig.12A)

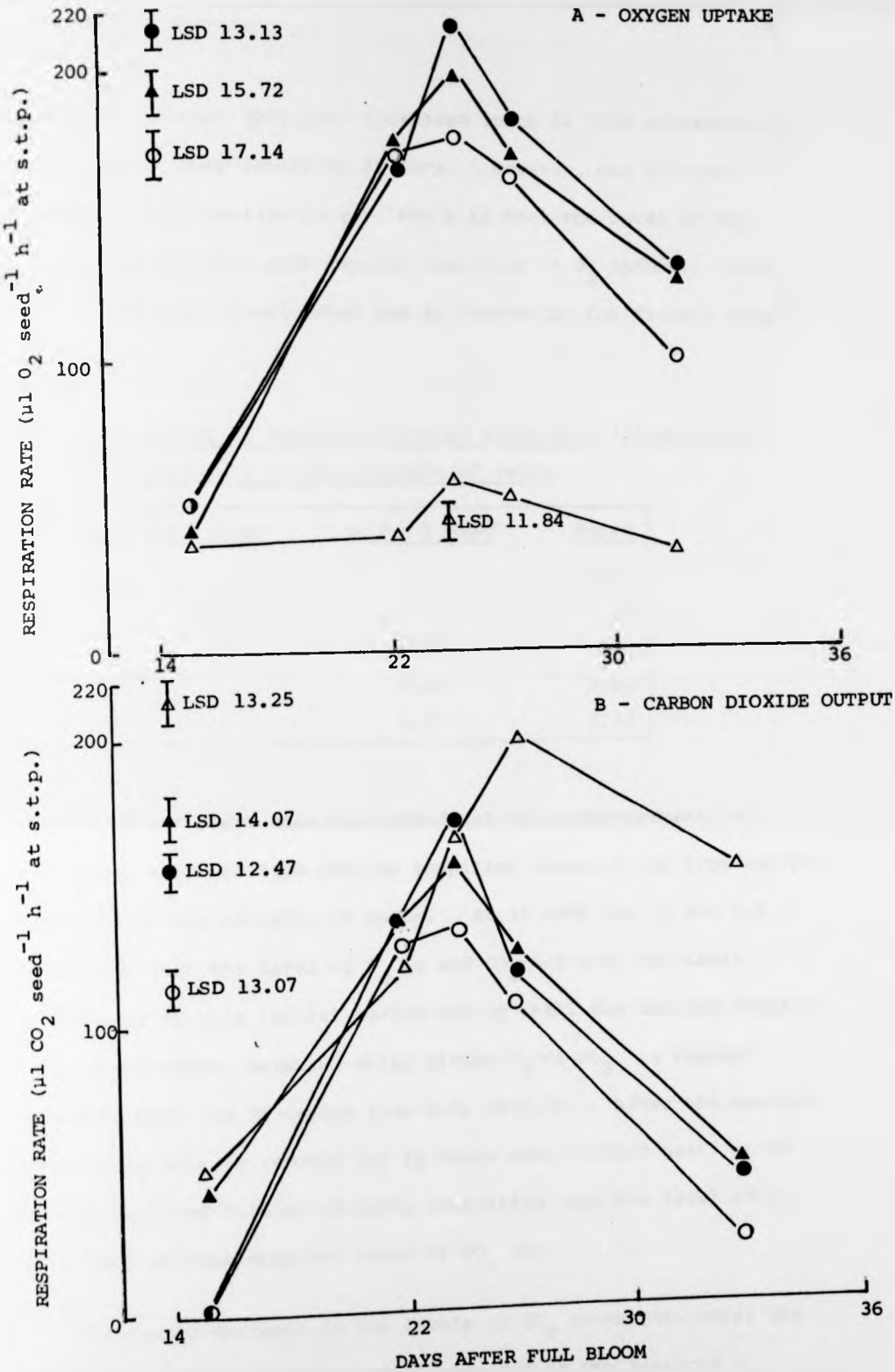

FIG. 12 Oxygen uptake - A and Carbon Dioxide output - B of 5 replicates of 5 embryos set to respire: normally (O), in the presence of water (●), in the presence of KCN (Δ) or in the presence of SHAM (▲).

and the CO_2 output (Fig.12B) increased up to 24 DAFB subsequently declining to lower levels by 32 DAFB. However, one striking feature of this decline in gas levels is that the level of CO_2 output declines much more rapidly than that of O_2 uptake. This can be seen more clearly when the RQ (Table 10) for freshly sampled

TABLE 10

Changes in the RQ of freshly harvested seeds of different ages, measured directly or in the presence of water

Days After Full Bloom	Without Water	Water
15	1.0	1.0
22	0.73	0.78
24	0.72	0.78
26	0.62	0.62
32	0.21	0.33

embryos of different ages are worked out using measurements of both gases obtained from embryos respiring normally and from embryos respiring in the presence of water. At 15 DAFB the RQ was 1.0 indicating that the level of O_2 in and CO_2 out were the same. However, after this initial period and up until the maximum respiration rate (whether measured using either O_2 or CO_2) is reached around 24 DAFB the RQ varies from 0.72 to 0.78. After the maximal respiration rate is reached the RQ drops even further until it is between 0.21 and 0.33 by 32 DAFB, indicating that the level of O_2 in is much greater than the level of CO_2 out.

This rapid decrease in the levels of CO_2 given out, after the peak of respiratory activity, when compared to the level of O_2 uptake could be explained in several ways. Firstly, there may be more than one respiratory pathway in operation over the time span

studied and the relative usage of these pathways may change during development. Secondly, the embryos may be able to photosynthesise thus fixing a proportion of the CO_2 released during respiration and thirdly, there may be more than one respiratory susbtrate used by the embryo at different stages of its development.

In order to try and assess what respiratory pathways may be operating two specific inhibitors were used; potassium cyanide, which inhibits the activity of the terminal oxidase cytochrome C and SHAM, which is believed to interfere with an "alternate respiratory pathway", insensitive to cyanide (Wilson and Bonner, 1971; Yentur and Leopold, 1976). Five replicates of five freshly sampled embryos were used at each sampling time, again using embryos over the period 15 to 32 DAFB. The inhibitors were supplied as 1mM solutions and, as the presence of water has already been shown to have an inhibitory effect on seed respiration a water control was used. It can be seen that potassium cyanide (Fig.12A) markedly inhibited the level of 0_2 uptake at all the sampling times used. SHAM on the other hand, showed a similar pattern of change to that of the water control (Fig.12A), i.e. 0_2 uptake rising to a maximal level at 24 DAFB subsequently falling to a lower level. This indicates that the SHAM had no inhibitory effect on the respiratory pathways involved. When the CO_2 uptake was measured, again the SHAM treated seeds (apart from the initial reading at 15 DAFB) showed a similar effect to that of the water control (Fig.12B) i.e. CO_2 output rose to a maximal level at 24 DAFB subsequently falling to low levels. The effect of potassium cyanide (Fig.12B) was similar to that of SHAM until the peak in respiration rate was reached, i.e. apart from the initial reading

at 15 DAFB when the CO_2 level was boosted slightly, the pattern of CO_2 output shown by the potassium cyanide treated embryos was similar to the pattern shown by both the SHAM treated embryos and the water controls. However, after the peak in respiratory activity at 24 DAFB, the pattern shown by the potassium cyanide treated embryos changed - the CO_2 output of the potassium cyanide treated embryos continued to increase until 26 DAFB then began to decline slowly. This was in contrast to the decline in CO_2 output.

In a second harvest series covering 19 to 34 DAFB the effect of potassium cyanide was again examined. As before, potassium cyanide almost completely inhibited 0_2 uptake throughout the period studied (Fig.13A). There were no significant differences between the rates of 0_2 uptake measured with potassium cyanide present in either the light or in the dark at any of the sample times used. In both the dark and in the light at 19 and 25 DAFB there were no differences between the levels of CO_2 output however, the presence of potassium cyanide appears to have an inhibitory effect on CO_2 output at this time (Fig.13B). As development proceeds this effect is much less marked and disappears by 29 DAFB. The levels of CO_2 at both 29 and 34 DAFB show the same tendency as in the first harvest series, namely that after the physiological activity of the embryos declines when measured by either 0_2 uptake or CO_2 output the presence of potassium cyanide results in an increase in the level of CO_2 released.

Thus this work with respiratory inhibitors would suggest that over the time period studies the predominant respiratory pathway involved is via the TCA-terminal oxidase system. The inability of SHAM to have any noticeable effect on either the CO_2 output or the

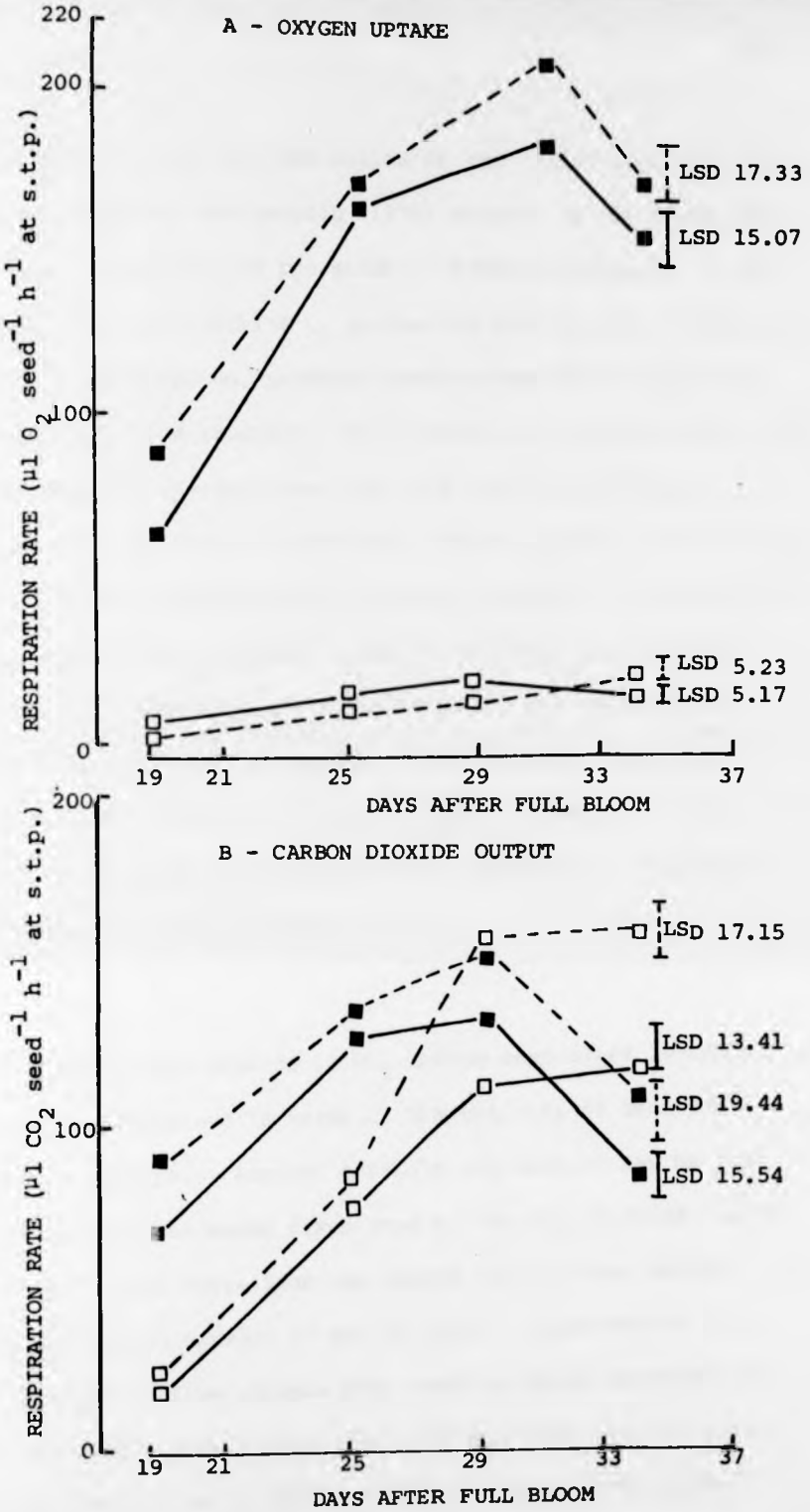

FIG.13 Oxygen uptake - A and Carbon Dioxide output - B of 5 replicates of 5 embryos set to respire in the presence of water (■) or in the presence of KCN (□) in either the light (——) or in the dark (---)

0_2 uptake tends to rule out the action of the "alternate respiratory pathway" which Yentur and Leopold (1976) suggest is the first pathway utilized in germinating pea seeds. Potassium cyanide, on the other hand, markedly inhibits 0_2 uptake but not CO_2 output suggesting a terminal oxidase system, possibly cyctochrome oxidase operates throughout the period studied. It is worth noticing that (Fig.13A) although potassium cyanide does have this inhibitory effect, 0_2 uptake is never completely inhibited. There appears to be a basic level of 0_2 uptake unaffected by potassium cyanide. The effect of potassium cyanide on CO_2 uptake after 24 DAFB may simply be due to the reduction in physiological activity being the result of a reduction in efficiency in the TCA cycle while the activity of glycolysis is not so immediately effected. However, an alternative explanation of this may be that there is a Pentose Phosphate Pathway also involved, the activity of which is stimulated were biosynthetic reactions are particularly active producing CO_2.

Thus, the marked decline in CO_2 uptake seen after 24 DAFB cannot just be explained in terms of the activity of different respiratory pathways. Another possible explanation may be that photosynthesis in pea seeds fixes some of the CO_2 released during respiration. This suggestion was tested in a harvest series covering the period between 19 and 34 DAFB. Measurements using five replicates of five embryos were used to obtain measurements of both CO_2 output and 0_2 uptake in both the light and the dark. In this harvest series 0_2 uptake and CO_2 output, in the light, increased until 29 DAFB after which it declined (Fig.13A). A similar pattern of changes in both 0_2 uptake and CO_2 output was also found when measurements were made in the dark (Fig.13B).

Not only was the pattern of changes in CO_2 and O_2 levels the same in the light and the dark but the volumes of both gases, especially CO_2 did not differ significantly in both the dark and light. This would suggest that there is not an active photosynthetic system which could explain the greater reduction in CO_2 levels found in developing pea seeds over the period studied. In this harvest series, as in the first harvest series described in this section, the level of CO_2 output declined more rapidly than the level of O_2 uptake (Fig.13A, Fig.13B). As before this can conveniently be expressed by the RQ and again, the RQ initially found at 19 DAFB is 1.0 but this has declined by 34 DAFB to 0.53 in the light and 0.62 in the dark (Table 11). The RQ can be taken as an indication of the nature of the respiratory substrate being utilized. Thus the changes in RQ suggest that during the period studied there are changes in the substrate being respired.

TABLE 11

Changes in the RQ of freshly harvested seed of different ages, measured in the light and in the dark

Days After Full Bloom	Light	Dark
19	1.0	1.0
25	0.78	0.79
29	0.71	0.73
34	0.53	0.62

Thus this section suggests that there appears to be only one respiratory pathway predominantly involved during the period of development studies and that this pathway is an aerobic pathway which is strongly inhibited by potassium cyanide. It has also been shown that not only is there a decline in O_2 uptake but that

there is also a decline in CO_2 uptake which roughly parallels that of O_2 uptake after the peak of respiratory activity is reached. Furthermore, although there are similar changes in CO_2 and O_2 levels the CO_2 level decreases more rapidly than the O_2 level. The results in this section suggest that this rapid decrease in CO_2 uptake is not attributable to the operation of a photosynthetic system but to a change in respiratory substrate.

Section 3 : Phospholipid changes in developing seeds

In the first section of the results in this thesis it was shown that the ability of desiccated seeds to retain solutes improves with age of the seed at harvest. Table 12 relates not only the viability of seeds after desiccation, as measured by

TABLE 12

The mean electrical conductivity of soak water (μmhos cm^{-1} g^{-1} d.wt.) and the staining categories of pea seeds (based on the staining of the abaxial surface of the cotyledons with tetrazolium chloride) sampled at different times after full bloom

DAFB	Cotyledon Staining Categories				Embryo Axis Complete Staining	24h conductivity μmhos cm^{-1} g^{-1} d.wt.
	I	II	III	IV		
17	-	-	-	15	-	3564
22	-	-	-	15	-	3097
23	-	3	3	9	-	2483
25	-	-	12	3	-	1850
29	9	3	3	-	7	1498
31	9	4	2	-	13	676
36	15	-	-	-	15	454

I = complete staining
II = < 50% unstained
III = > 50% unstained
IV = no staining

complete tetrazolium staining of the embryo axis and embryo condition, as estimated by conductivity measurements (these have already been presented in Fig.3B in section 1) but also relates the amount of dead tissue present on the cotyledons (measured by the lack of tetrazolium staining of the cotyledons) with the level of leaching from the embryo. From this (Table 12) two important observations can be made. Firstly, that the decline in

the leaching of solutes was a feature of both dead seeds i.e. of seeds at 17, 22, 23 and 25 DAFB and of live seeds i.e. at 29, 31 and 36 DAFB and secondly, that there is a reduction in leakage from seeds of different ages which have approximately the same amount of living tissue present i.e. 29 and 31 DAFB. This suggests that there was a continuous improvement in the retention of solutes by desiccated seeds with time, irrespective of the amount of living tissue present. Since the permeability of a cell and thus its capacity for solute retention is basically controlled by the state of membrane structures such as the plasmalemma it was thought that during the development of a seed changes in membrane structure might occur which would render the seed capable of withstanding dehydration in such a way that solutes would be retained more efficiently during imbibition. One chemical constituent of membranes that might reflect these sort of changes is the phospholipid component of the membranes.

Phospholipids were examined in three sets of circumstances. Firstly,changes in phospholipids were measured during the normal course of development in freshly harvested, succulent seeds. Secondly, phospholipids were measured in succulent seeds that had been subjected to three days high humidity storage which would be expected to advance the time at which seeds were able to withstand desiccation. Thirdly, the phospholipid content of dried seeds of different ages was also measured in an attempt to relate the changes which might be necessary prior to desiccation with the state of membranes in the desiccated, viable seed. In all circumstances measurements of respiration rate, dry weight, embryo condition (conductivity) and viability (tetrazolium staining of the

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embryo axis) were made so that any change in phospholipid content of the seeds could be related to the physiological changes occurring during seed development.

Phospholipid changes during normal development

Before looking at changes in phospholipid levels during development it is important to establish the timing of the important physiological changes which occur. This was done in the two harvest series used. In the first harvest series (Fig.14A) the respiration rate increased up to 26 DAFB after which it declined. Dry weight increased until 29 DAFB. Embryo condition as measured by a reduction in the 24h conductivity improved significantly throughout the period studies. However, seeds first showed viability after desiccation at 26 DAFB which coincided with the peak in respiration rate. In the second harvest series (Fig.14B, open symbols) dry weight increased throughout. Respiration rate increased to a maximal level at 25 DAFB, however it did not decrease significantly until 30 DAFB. As in the first harvest series, embryo condition improved throughout but viability of seeds after desiccation did not appear until 30 DAFB when the respiration rate had declined significantly. It can therefore be said that seeds in the first harvest series are physiologically more mature than seeds in the second harvest series. Thus the ability of seeds to withstand desiccation developed around 26 DAFB in the first harvest series and around 30 DAFB in the second harvest series. If there are any chemical changes in membrane composition which are necessary for the development of the ability to withstand desiccation then they would be expected to occur on or before 26 DAFB in the first harvest series or 30 DAFB in the second harvest series.

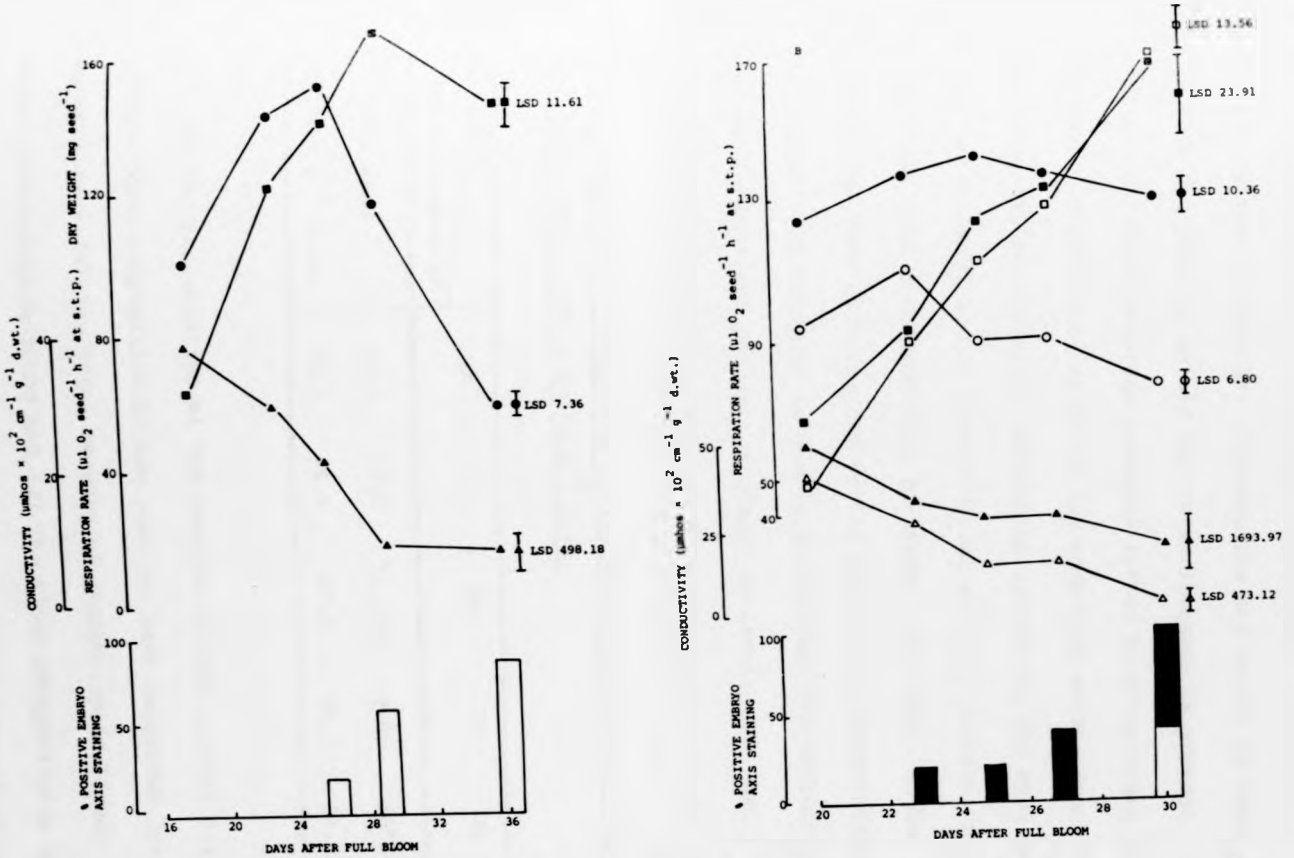


FIG.14 Changes in respiration rate (O ●), dry weight (□ ■) (mean of 5 replicates of 5 seeds) the 24h conductivity of steep water from desiccated seeds (Δ ▲) and the tetrazolium staining of the embryo axes (mean of 10 single seeds) during normal development (O, □, Δ) and after storage for 3 days under ≈ 100% rh (●, ■, ▲) in A - the harvest series 1 and B - harvest series 2.

The phospholipid content of succulent seeds was examined by extracting phospholipids from three replicates of three embryos of different ages in both harvest series. Phospholipids are expressed per seed; as are most of the results in this thesis, for two reasons - firstly, although the dry weight of seeds increases with age this is mainly due to an increase in storage reserves such as starch and storage proteins rather to an increase in cell numbers. Furthermore at about the same time as the peak in physiological activity the membranous systems of the seed were fully established and as physiological activity declines membrane structures tend to become less distinct. Secondly, in Table 13 it can be seen that changing the way in which total phospholipids (quantitatively measured as μ mole phosphorus) are expressed does not radically alter the pattern of change in levels with age.

TABLE 13

Total phospholipid phosphorus during normal development expressed as μ mole seed^{-1} and μ moles g^{-1} d.wt.

DAFB / Total phosphorus	18	23	26	29	36
μ mole seed^{-1}	2423	5326	12,302	9008	8552
μ mole g^{-1} d.wt.	38.3	44.9	87.3	55.1	58.13

In the presentation of the results of both harvest series the phospholipid composition of the seed has been separated into three categories - (a) the total amounts of phospholipids found; (b) the major phospholipids found and (c) the minor phospholipids which includes both the phospholipids which were not identified and the phosphorus remaining at the origin.

(a) Total levels of phospholipids

In the first harvest series the total amounts of phospholipids extracted from the succulent seeds increased by approximately 300% between 23 and 26 DAFB (Fig.15A) thereafter the total levels of phospholipids did not alter significantly. In the second harvest series the total phospholipid content increased throughout with significant increases in levels between 20 and 23 DAFB and 25 and 30 DAFB (Fig.15B). Thus, there were significant increases in total levels of phospholipids when there was a peak in respiratory activity and seeds first developed the ability to withstand desiccation i.e. at 26 and 30 DAFB in the first and second harvest series respectively.

(b) The major phospholipids

In both harvest series phosphatidyl choline (PC) was the main phospholipid species found with phosphatidy inositol (PI) and phosphatidyl glycerol (PG) also being found in relatively large amounts. Of these major phospholipids the pattern of PC and PI found in seeds in both harvest series showed similar patterns of changes as that of the total levels. In the first harvest series there was a significant increase in levels of PC and PI between 23 and 26 DAFB which was immediately prior to the development of the ability to withstand desiccation by the seed (Fig.15A). In the second harvest series there were significant increases between 20 and 23 DAFB and more importantly between 25 and 30 DAFB (Fig.15B). The pattern of PG in both harvest series differs slightly from the pattern of changes found in the total level, in PC and in PI. As before there are increases in the levels of PG before the peak in physiological activity, shown by the seed (Fig.15). In the first

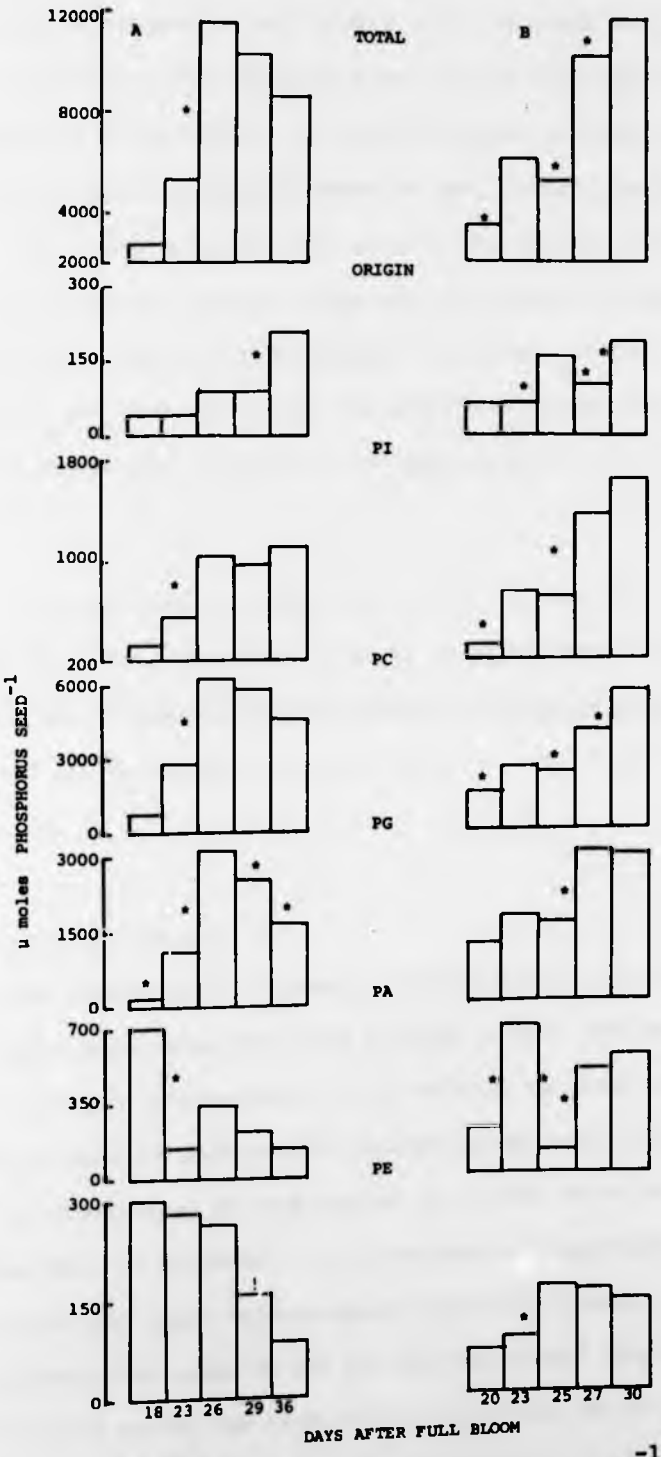


FIG.15 Changes in the amounts (μ moles phosphorus seed^{-1}) of the total phospholipids, the known phospholipids and phosphorus remaining at the origin found in succulent seeds during normal development in A - harvest series 1 and B - harvest series 2. Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by placing of a * beside one histogram and above the other.

harvest series this was shown by significant increases in PG in seeds between 18 and 26 DAFB while in the second harvest series by a significant increase between 25 and 27 DAFB. It is after this peak in physiological activity that the pattern of PG diverged from the patterns already established. In the first harvest series the level of PG significantly declined between 26 and 36 DAFB despite an increase in seed viability over this period (Fig.15A). The levels of PG in the second harvest series did not show a significant decline after the peak in physiological activity. It is possible that the differences may be connected to the differences in the state of physiological maturity of seeds in both harvest series.

Thus of the major phospholipids, levels of PC, PI and PG increased significantly either just prior to or at the same time as there was a peak in physiological activity, a reduction in 24h conductivity and the development of the ability of seeds to withstand desiccation.

(c) The minor phospholipids

Of the known phospholipids measured, phosphatidic acid (PA) and phosphatidyl ethanolamine (PE) were present in much smaller amounts than the major phospholipids. In addition to these minor phospholipids a number of unidentified phospholipids were also observed and are considered in this section as is the amount of phosphorus remaining at the origin. Of the minor phospholipids components (PA and PE) there did not appear to be any strong similarities between the seeds of the two harvest series (Fig.15). In the first harvest series the level of PA was highest at 18 DAFB

after which it declines significantly to a relatively constant level. In the second harvest series the level of PA increased significantly from 20 to 23 DAFB. After 23 DAFB the PA levels vary considerably but there was an overall significant decrease in levels between 23 and 30 DAFB. In the first harvest series the levels of PE showed a significant decrease between 26 and 30 DAFB but the seeds of the second harvest showed a significant increase between 23 and 25 DAFB after which levels remain constant. These apparent inconsistencies between the two harvest series may again be connected with the differences in the state of physiological maturity of the seeds of both harvest series. Thus PA appears to decrease significantly before the seed first develops the ability to withstand desiccation but the connection of PE with the development of this ability still remains obscure.

Apart from these known minor phospholipids, the amount of phosphorus remaining at the origin is also of interest. The phosphorus at the origin can be considered to be a measure of a combination of any phospholipids which may be too polar to run in the solvent system used and any phosphorus which may be released due to phospholipid degradation. In the first harvest series, there was a significant increase in phosphorus at the origin between 29 and 26 DAFB, which was after both the peak in respiratory activity and the development of the ability to withstand desiccation during development (Fig.15A). In the second harvest series there was a significant increase in levels between 23 and 30 DAFB which was before both the peak in respiratory activity and the development of the ability to withstand desiccation (Fig.15B).

During the normal course of development three unidentified

phospholipids were found in both harvest series (U_4, U_6 and U_7) (Fig.16). In the first harvest series U_4 was present in very low levels which did not differ significantly while U_6 and U_7 are found only at 36 DAFB. In the second harvest series U_4 was present in substantially larger amounts and gradually increased with age producing an overall significant increase between 20 and 30 DAFB. U_6 and U_7 appeared 27 DAFB and continued to increase in amount until 30 DAFB. What the significance of these changes might be is uncertain and will be discussed later.

In summary, the investigation of different phospholipid species during normal development when interpreted in connection with other physiological changes would suggest that a low level of leakage, a peak in respiratory activity and the seed first being able to withstand desiccation may be connected with increased in the total levels of phospholipid present in the seed, in PI, PC and PG. PA tends to decrease before the development of the ability to withstand desiccation but changes in the other minor phospholipid, the unknown phospholipids and phosphorus at the origin appear to be much more tenuously connected with this development of the ability to withstand desiccation by seeds.

Phospholipid changes in succulent seeds stored under high humidity conditions

Having established that some chemical changes in phospholipids did occur during normal development, even though their role may not be very clear, it was thought desirable to see what phospholipid changes would occur when a seed prematurely develops the ability to withstand desiccation. In the Section 1 of the Results in this thesis it has been shown that by holding seeds in high humidity

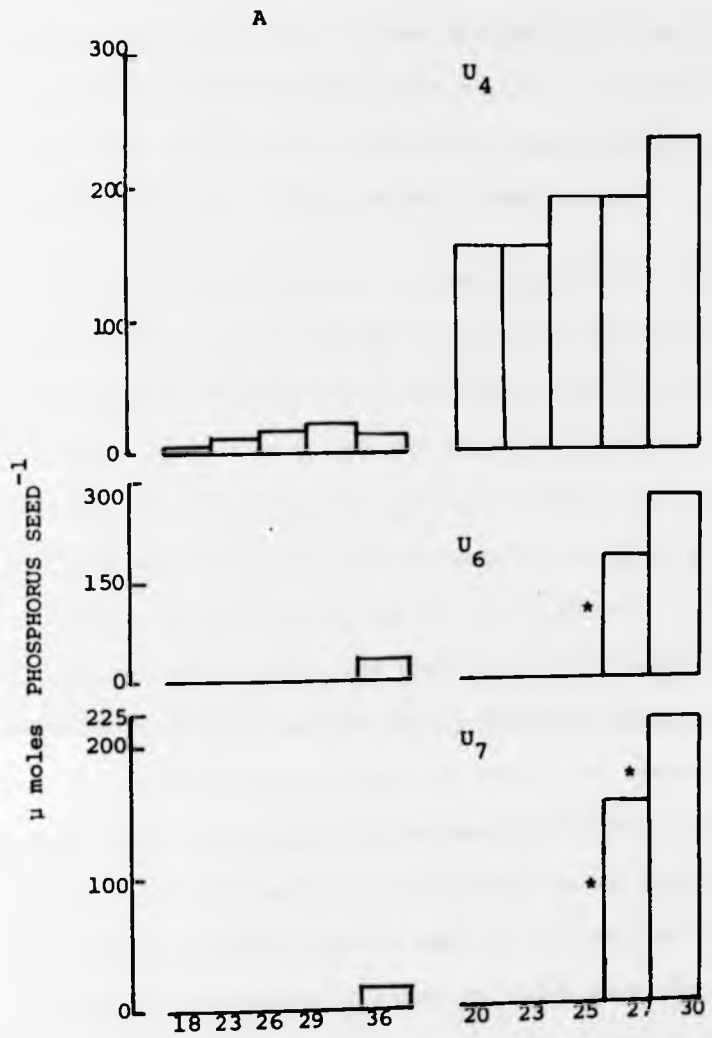


FIG.16 Changes in the amounts (μ moles phosphorus seed^{-1}) of the unidentified phospholipids found in succulent seeds during normal development in A - harvest series 1 and B - harvest series 2. Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by the placing of a * beside one histogram and above the other.

storage they can be induced to develop the ability to withstand desiccation earlier than seeds of the same age sampled freshly from the plant. This being so, if some phospholipid changes are necessary for the development of the ability to withstand desiccation then they should also be induced by high humidity storage. This was investigated in the second harvest series.

Again, before any changes in phospholipids can even be considered the effects of high humidity storage on other physiological processes must be established in the harvest series used. After holding seeds of different ages for three days at 100 rh the respiration rate (Fig.14B) was reduced on average by 32% and the 24h conductivity of the desiccated seeds was reduced by 18% at 20 and 23 DAFB rising to a reduction of 89% by 30 DAFB. Finally, and perhaps most significantly, the viability of the seeds as estimated by tetrazolium staining of the embryo axis was observed as early as 23 DAFB in the humid stored seed with 20% of the seeds being viable compared to all the freshly sampled seeds being non-viable. By 30 DAFB 100% of the humid stored seed were viable compared to the 20% viability of freshly sampled seed of the same age (Fig.14B). The phospholipid components of succulent seeds were investigated before and after high humidity storage. Again the phospholipid results will be considered under three headings.

(a) Total phospholipids

When the total phospholipid levels were examined (Fig.17) a similar pattern of phospholipid changes was found in those seeds which had been freshly sampled (Fig.17A) and the stored seed which had been kept for three days under 100% rh (Fig.17B). There was

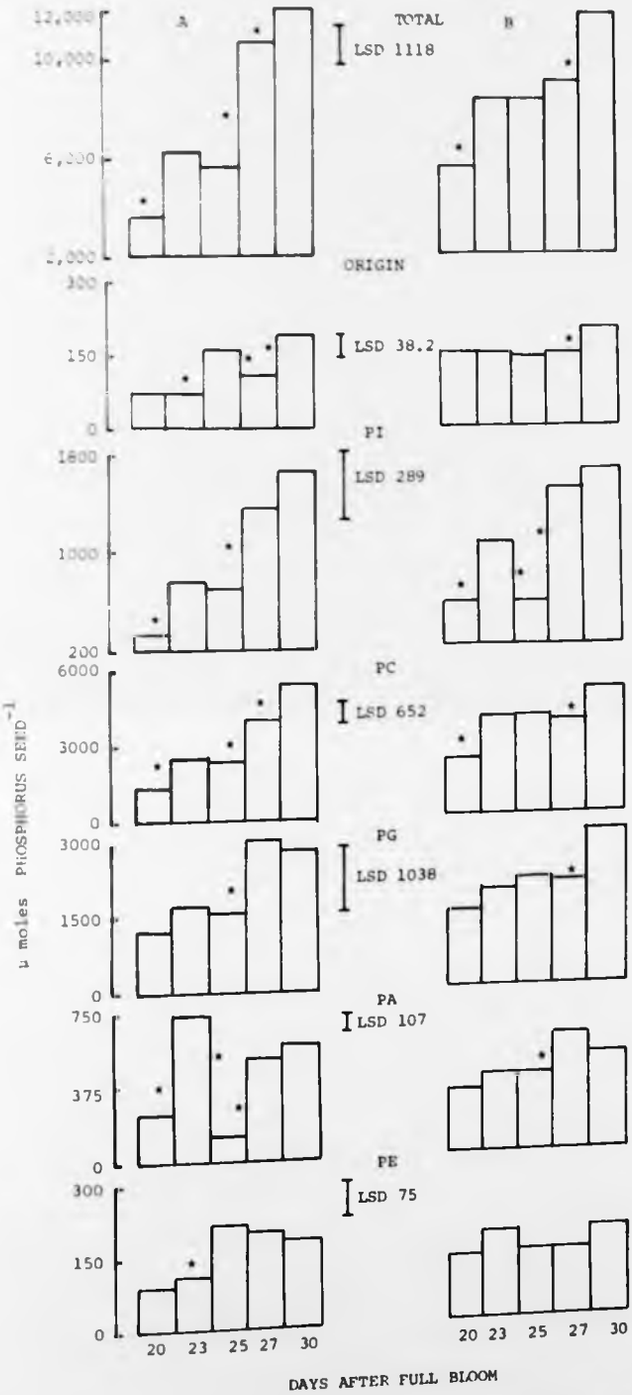


FIG.17 Changes in the amounts (μ moles phosphorus seed^{-1}) of total phospholipids, the known phospholipids and phosphorus remaining at the origin from succulent seeds harvested freshly (A) and stored for 3 days under = 100% rh (B). Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by the placing of a * beside one histogram and above the other.

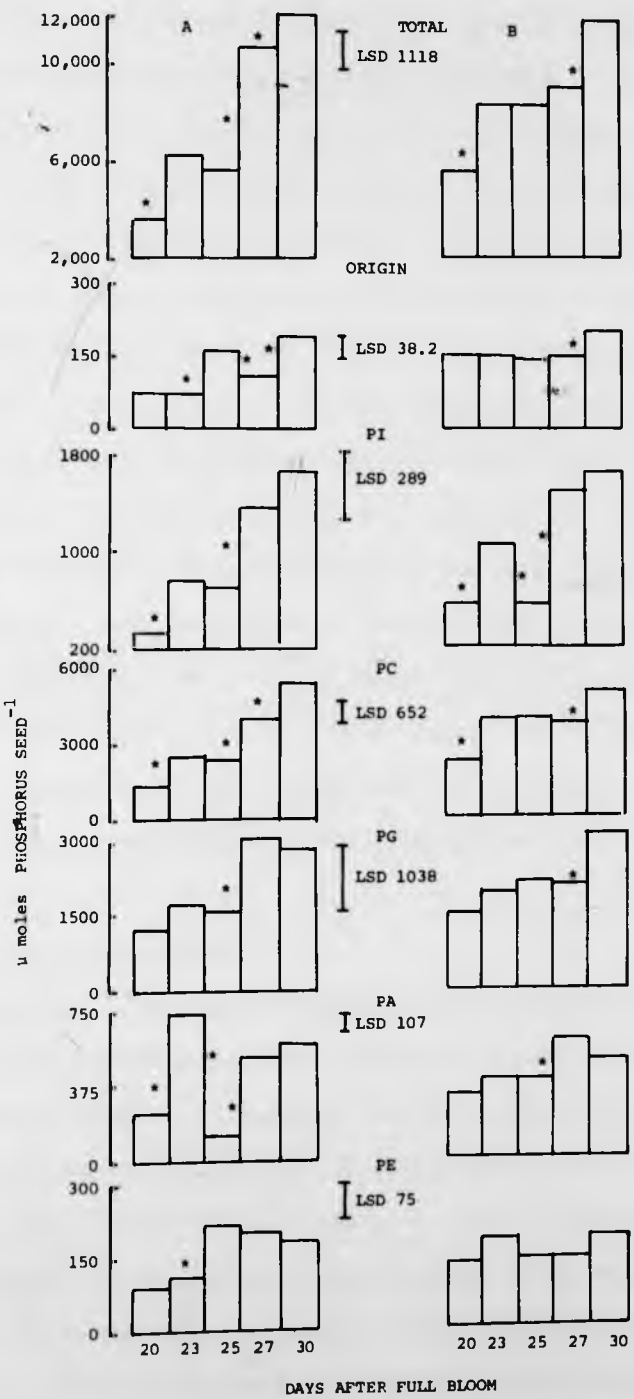


FIG.17 Changes in the amounts (µ moles phosphorus seed^{-1}) of total phospholipids, the known phospholipids and phosphorus remaining at the origin from succulent seeds harvested freshly (A) and stored for 3 days under = 100% rh (B). Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by the placing of a * beside one histogram and above the other.

an early and significant rise in total phospholipid levels between 20 and 23 DAFB in both fresh and stored conditions (Fig.17 A and B). At 23 DAFB there were no viable seeds in the freshly sampled seed but there was 20% viability in the humid stored seed. In the fresh seed there was a significant rise in total phospholipid levels between 25 and 30 DAFB which would be prior to the development of 20% viability at 30 DAFB for these seeds. There was also a significant increase in total phospholipid levels between 27 and 30 DAFB in the stored seed by which time 100% viability of these seeds had been achieved. It is also of note that (Table 14) the total levels of phosphorus found in the stored seed were significantly higher than the levels found in the fresh seed. However, by 30 DAFB there is no significant difference between the levels found in the two treatments. Thus investigation of the phospholipid content of seeds stored under 100% rh which prematurely develop the ability to withstand desiccation tend to further support the findings during normal development that total phospholipd levels increase prior to the development of the ability to withstand desiccation.

(b) The major phospholipids

As before PC, PI and PG are the predominant lipids found. Again PC showed a similar pattern of changes to those of the total levels, with a significant increase in levels between 20 and 23 DAFB in the fresh seed and between 20 and 23 DAFB and 27 and 30 DAFB in the stored seeds (Fig.17A and B). Again (Table 13), until 27 DAFB the level of PC is significantly higher in the stored seed. The pattern of PG for both treatments differs slightly from the total levels in that there was a significant increase in PG between 25 and 27 DAFB in the fresh seed which preceded the development of

TABLE 14

Analysis of changes (based on Duncan's Multiple Range Test) in the main phospholipids extracted from fresh and dried pea seeds, as a result of three days storage at 100% rh $p \leq 0.05$

Days After Full Bloom		Phosphorus at Origin	PI	PC	U_4	PG	PA	PE	Total
Fresh	20	***	NS	*	NS	NS	*	NS	**
	23	***	NS	***	NS	NS	***	NS	***
	25	NS	NS	***	NS	NS	*	*	***
	27	NS	NS	NS	NS	*	NS	NS	**
	30	NS	NS	NS	NS	NS	NS	NS	NS
	Overall	***	NS	***	NS	NS	NS	NS	***
Dried	20	NS	NS	NS	*	NS	NS	NS	NS
	23	NS	NS	NS	NS	NS	NS	NS	*
	25	NS	*	NS	*	NS	***	*	*
	27	NS	NS	***	NS	NS	NS	NS	***
	30	NS	NS	**	NS	NS	*	NS	NS
	Overall	NS	NS	***	NS	NS	***	NS	NS

viability at 30 DAFB. In the stored seed PG did not significantly increase until 30 DAFB. Again, unlike either the total levels or the levels of PC, the levels of PG found in both the fresh and stored seed did not differ significantly except at 27 DAFB which was before the fresh seed developed the ability to withstand desiccation but after the stored seed had developed this ability (Fig.17A and B). PI on the other hand, showed an early increase between 20 and 23 DAFB in both fresh and stored seed and between 25 an 27 DAFB in the fresh seed and between 23 and 27 DAFB in the stored seed. However, there were no significant differences between the amounts of PI in the fresh and stored seed. Thus, only changes in PC levels in seeds which prematurely developed the ability to withstand desiccation corresponded with the changes in the major phospholipid levels found during normal development.

(c) The minor phospholipids

There was a significant increase (Fig.17A) in PA levels between 20 and 23 DAFB in the fresh seed. After this the levels varied considerably but there was an overall decrease in levels between 23 and 30 DAFB when these seeds first developed the ability to withstand desiccation. In the stored seed there was a significant increase in PA levels between 25 and 27 DAFB and significant differences between the levels of PA found at 20, 23 and 25 DAFB in both fresh and stored seed (Table 14) but by 27 DAFB there were no significant differences between the two conditions. These wide fluctuations would tend to suggest that fluctuations in PA levels do not have any obvious links with the physiological events investigated namely, with either a reduction in respiration rate or conductivity or with the development of the ability to withstand

desiccation. The levels of PE in the stored seed increased significantly between 23 and 25 DAFB in the fresh seed (Fig.17A). However, not only were there no significant changes in PE levels with age in the stored seed but there were no significant changes between PE levels in the fresh and stored seed except at 25 DAFB. Again it would appear that PE levels may not be connected with the other physiological events measured.

In the freshly harvested seed phosphorus at the origin showed a significant increase between 23 and 30 DAFB but this increase occurred later in the stored seed between 27 and 30 DAFB (Fig.17A and B). The levels of phosphorus at the origin were significantly higher at 20 and 23 DAFB in stored seed than in fresh seeds. Again the connection of phosphorus at the origin and the main physiological events leading to the development of the ability to withstand desiccation by seeds is not clear.

Of the unidentified phospholipids U_4, U_6 and U_7 were the main phospholipids found (Fig.18A and B) U_4 showed no change either with age or with storage in the fresh and stored seed. In the fresh seed U_6 and U_7 were only found after 25 DAFB and the increase significantly up to 30 DAFB which preceded the development of viable seeds at 30 DAFB. In the stored seed U_6 and U_7 were both found from 20 DAFB. U_6 tended to increase with age while U_7 increased significantly between 20 and 23 DAFB (viable seeds were first found at 23 DAFB) and again between 25 and 27 DAFB but there was a fall between 27 and 30 DAFB. Again the levels of U_6 and U_7 were significantly higher in the stored seed which showed viable seeds by 23 DAFB than in the fresh seed however, by 30 DAFB, when some viable seeds are found in the fresh seed there were no significant

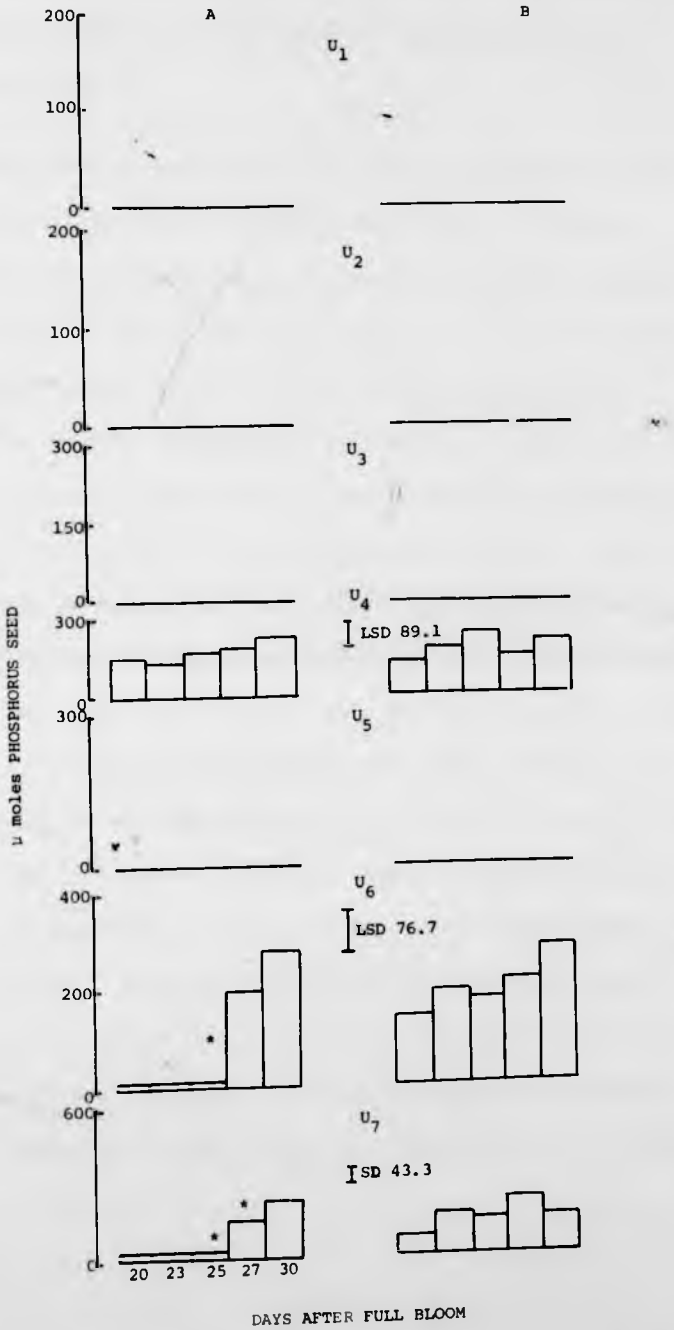


FIG.18 Changes in the amounts (μ moles phosphorus) of the unidentified phospholipids found in succulent seeds harvested freshly (A) and stored for 3 days under = 100% rh (B). Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by the placing of a * beside one histogram and above the other.

differences between the fresh and stored seed (Table 15). Two other unknown phospholipids were found, U_8 and U_9, however as they ran close together under the solvent front and have an extremely variable pattern of changes in succulent seeds they are only presented in Appendix 1.

It can generally be said that with increase in age of succulent seeds, which coincides with a continual improvement in embryo retention of solutes (a decrease in conductivity), whether they be sampled freshly or stored for three days at 100% rh, that there tended to be an increase in the total amount of phospholipids present, in the amount of phosphorus at the origin and in the PC, PI, U_6 and U_7 levels. The levels of PG, PA and PE tended not to show any consistent pattern. U_4 showed little change. When the effect of storage of succulent seeds, which would be expected to improve embryo condition and the ability to withstand desiccation, was examined generally, the levels of PI, PG and PE remain unaltered. Storage however, significantly increased the total phospholipid levels, the levels of PC, phosphorus at the origin U_6 and U_7. The effect of storage on PA levels was more complex with considerable variations being found but overall, storage had no significant effect on PA levels. Therefore, the phospholipid levels that tend to change under conditions where embryo condition leading to the development of the ability to withstand desiccation improve, are the total levels of phospholipids, PC, phosphorus at the origin, U_6 and U_7.

TABLE 15

Analysis of changes (based on Duncan's Multiple Range Test) in the unknown phospholipids extracted from fresh and dried pea seeds, as a result of three days storage at 100% rh $p \leq 0.05$

	Days after Full Bloom	U_1	U_2	U_3	U_5	U_6	U_7	U_8	U_9
Fresh	20	—	—	—	—	**	***	NS	NS
	23	—	—	—	—	***	***	NS	NS
	25	—	—	—	—	***	***	***	***
	27	—	—	—	—	NS	**	***	***
	30	—	—	—	—	NS	NS	***	NS
	Overall	—	—	—	—	***	***	***	***
Dried	20	NS	NS	***	NS	***	**	*	NS
	23	NS	NS	NS	NS	NS	**	NS	NS
	25	NS	*	NS	**	NS	NS	**	***
	27	NS	NS	NS	NS	NS	NS	NS	NS
	30	NS	NS	NS	NS	NS	***	NS	NS
	Overall	NS	**	*	NS	NS	NS	*	NS

Phospholipid changes in desiccated seeds

So far, this study has been concerned with what changes may occur in succulent seeds which might enable seeds to withstand drying however, it is not known whether the chemical composition of membranes is the same before and after drying. Physical changes do occur in membrane structure on dehydration (Simon, 1974) but no such information is available for chemical composition. If chemical changes do occur on drying this could have serious repercussions on the retention of solutes by the rehydrating seed on imbibition. This question was investigated, again in the second harvest series, under two sets of conditions. In the first, the phospholipid content of seeds sampled at times during normal development then desiccated under vacuum was estimated, and in the second, the phospholipid content of seeds of different ages, held for three days in high humidity storage prior to desiccation was estimated. Again explanation of these results falls into three categories. However, before looking at this, the most obvious difference between phospholipids extracted from seeds in the succulent state and from seeds in the dry state was that the levels of total phospholipids, PI, PC, PG and PA were markedly depressed in the dried seed (Fig.19). Despite this, the levels of phosphorus at the origin, PE, U_4 and U_6 found in the dried seed were similar to the levels found in the succulent seed while the levels of U_7 found in the dried seed were higher than those found in the succulent seed (Fig.19)

(a) Total Phospholipids

There was a significant increase in the total levels of phospholipids found between 23 and 30 DAFB in the dried fresh seeds

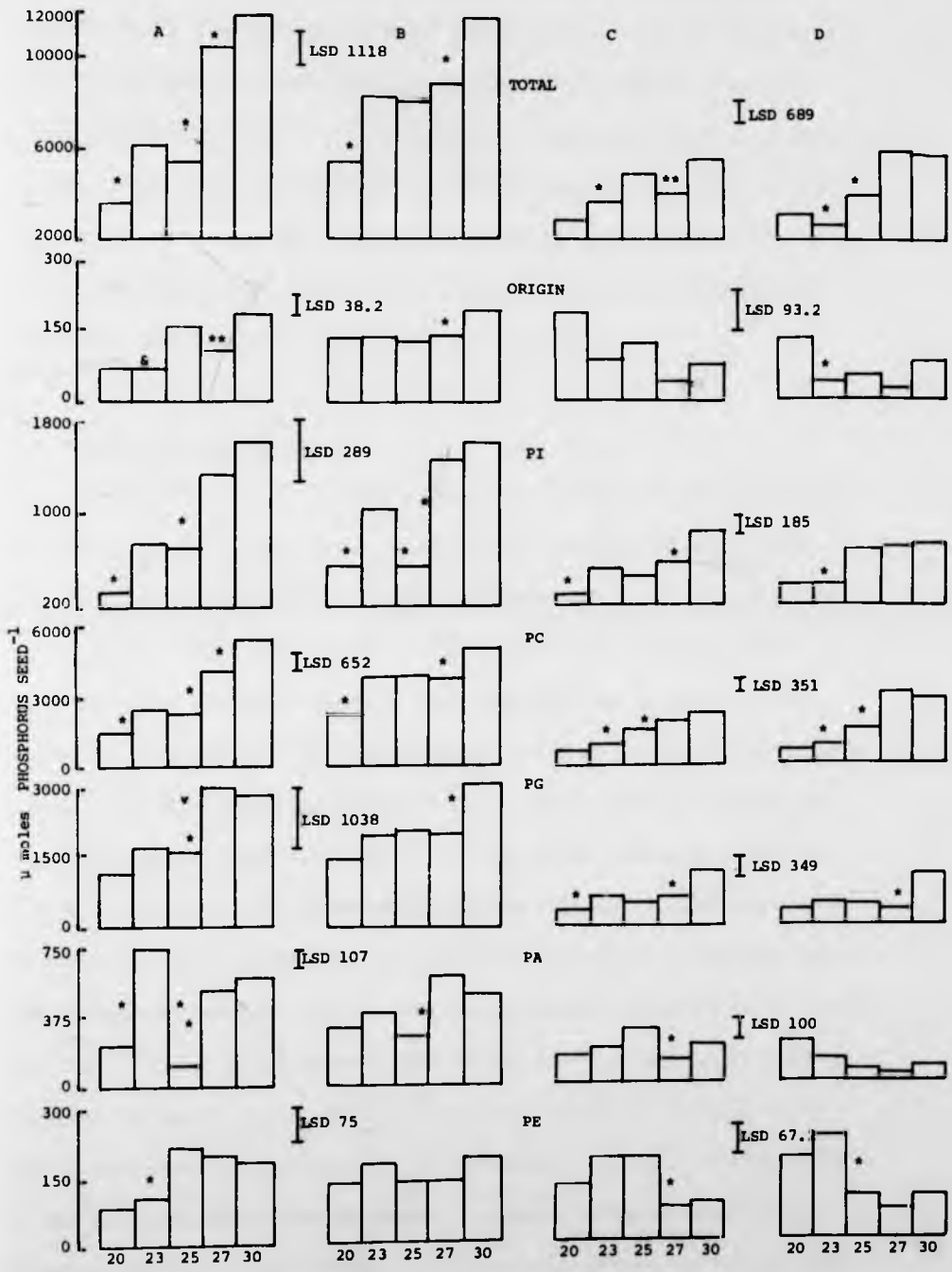


FIG.19 Changes in the amounts (µ mole phosphorus seed^{-1}) of the total phospholipids, the known phospholipids and phosphorus at the origin found in succulent seeds (A, B) and in dried seeds (C, D) harvested freshly (A, C) and stored for 3 days under = 100% rh (B, D). Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by the placing of a * beside one histogram and above the other.

(Fig.19C) and between 23 and 27 DAFB in the dried stored seed (Fig.19D). Furthermore, the effect of storage was only apparent at 23 and 25 DAFB when the total levels of phospholipids were significantly higher in the dried fresh seed at 23 and 25 DAFB but by 27 DAFB the situation was reversed. Again 20% of the dried fresh seeds were viable by 30 DAFB, so increases in total phospholipids follow the pattern already established in succulent seeds. The dried stored seeds showed 20% viability at 23 DAFB, thus in these seeds it would appear that viable seeds were found before the total phospholipids had begun to increase.

(b) The major phospholipids

Of the major phospholipids PC increased significantly between 23 and 27 DAFB in both dried fresh and dried stored seed; the levels of PC in the dried stored seed being significantly higher at 27 and 30 DAFB than those of the dried fresh seed (Fig.19). This may be a function of only 20% viability at 30 DAFB in the dried fresh seed but 100% viability at 30 DAFB in the dried stored seed. The levels of PI found in the fresh dried seed showed the same pattern of changes as those in the fresh succulent seed, i.e. PI levels increased significantly between 20 and 23 DAFB and again between 27 and 30 DAFB, the latter increase being immediately prior to the development of the ability to withstand desiccation by these seeds. In the dried stored seed PI levels increase significantly between 23 and 25 DAFB, which like the PC levels was just after the development of the ability to withstand desiccation was first found in these dried stored seeds. Again, storage appears to have no effect on the levels of PI found in either the dried fresh or dried stored seed (Table 14). The pattern of PG changes in

the dried fresh seed was similar to the pattern of PI changes in the same seed, i.e. the level of PG significantly increased between 20 and 23 DAFB and between 27 and 30 DAFB. However, in the dried stored seed PG levels increased significantly between 27 and 30 DAFB and again storage appeared to have no significant effect on the levels of PG found in either the dried fresh or stored seed (Fig.19). Thus again only the levels of PC seemed to be closely connected with the development of the ability to withstand desiccation, while desiccation itself seemed to mainly affect these major phospholipids by markedly depressing their levels.

(c) The minor phospholipids

Of the minor known phospholipids PE decreased significantly after 25 DAFB in the dried fresh seed and after 23 DAFB in the dried stored seed (Fig.19). Storage, with the exception of 25 DAFB did not have any significant effect on the PE levels in both types of seeds (Table 14). PA, on the other hand, decreased significantly between 25 and 27 DAFB in the dried fresh seed and between 20 and 27 DAFB in the dried stored seed. Storage also significantly decreased the levels of PA in the dried seed at 25 and 30 DAFB (Table 14). Again, the reduction in both PA and PE levels in the dried seed occurred on or before the seed was first able to withstand desiccation.

Phosphorus at the origin shows a similar pattern to that of PA and PE in that there is a tendency for the levels to decline with age in both dried fresh and stored seed with a significant decline between 20 and 30 DAFB in dried fresh seed and between 20 and 23 DAFB in dried stored seed. Although storage did not significantly

affect the level of phosphorus at the origin (Table 13) these declined in levels with age again preceded the development of the ability to withstand desiccation in both dried fresh and stored seed.

U_4, U_6 and U_7, which have been found in succulent seeds have also been found in dried seed (Fig.20). The levels of U_4 in the dried seed was extremely variable. U_6, unlike the succulent fresh seed was found in relatively high levels at 20 DAFB in the dried fresh seed. After 20 DAFB the level of U_6 in the dried fresh seed declined to relatively constant levels. The levels of U_6 in the dried stored seed did not vary much with age. Apart from the levels at 20 DAFB when the level of U_6 in the dried stored seed was significantly lower than in the dried fresh seed there were no significant differencesbetween the levels in the dried fresh and stored seed (Table 15). U_7 was again present in the dried fresh seed from 20 DAFB. In both the dried fresh and stored seed the levels of U_7 appeared to increase to a peak at 25 DAFB after which they declined. Again storage tended not to affect the levels of U_7 found in the dried seed. Of the other unknowns, U_8 and U_9 these decline with age in the dried seed (Appendix 1).

Apart from the unknown phosphorus containing lipids that were found both in the succulent and in the dried seed, four other unknowns were found in the dried seed. These have been called U_1, U_2 and U_3 which run on the TLC plate between the origin and PI, and U_5 which runs between U_4 and PG. These four unknown phospholipids fall into two groups - those which decreased with age and those which increased with age. In the first group U_1, U_2 and U_3 appeared to show the same pattern of change during development in

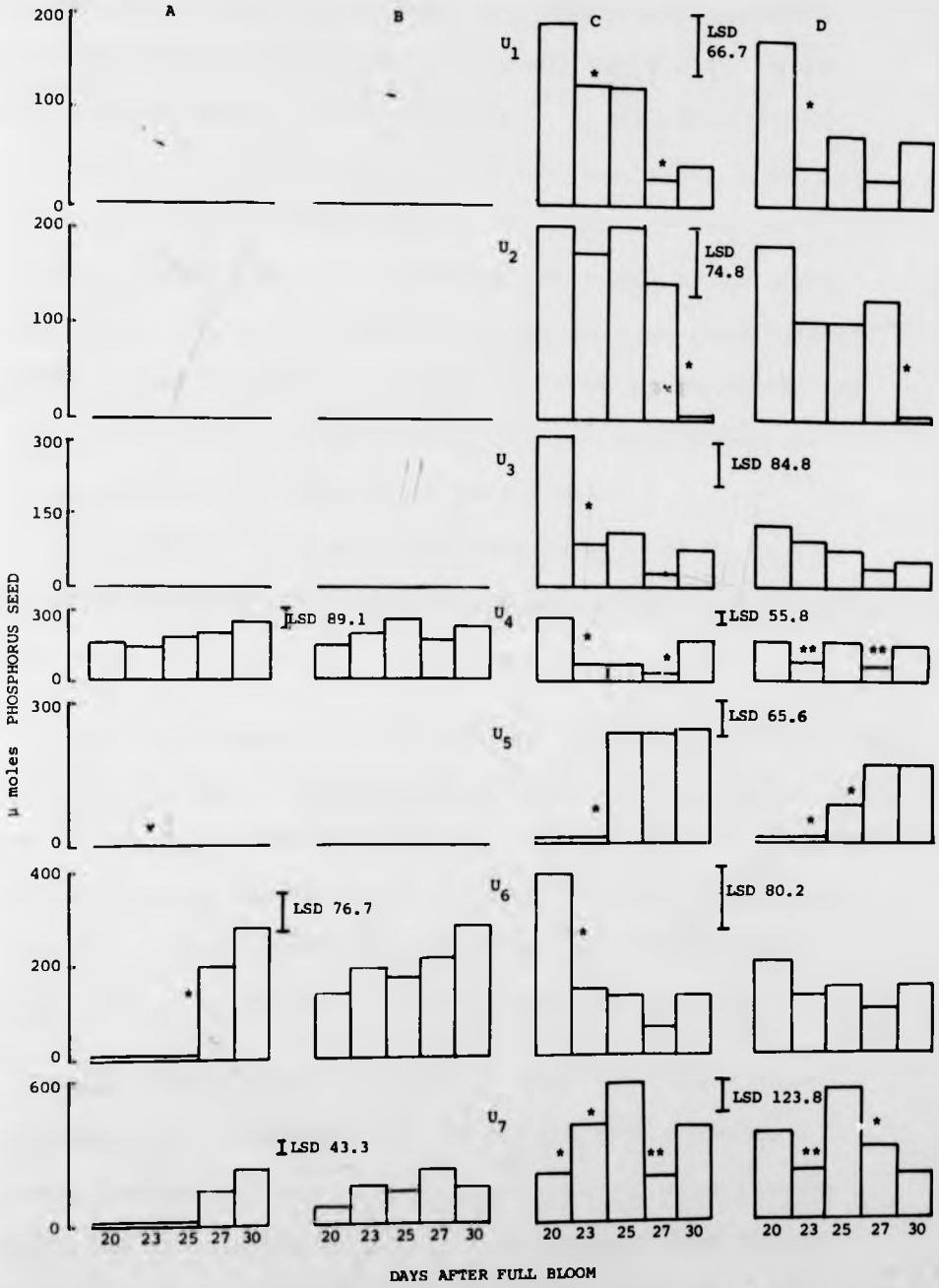


FIG.20 Changes in the amounts (μ moles phosphorus seed^{-1}) of the unidentified phospholipids found in succulent (A, B) and in dried seeds (C, D) harvested freshly (A, C) and after 3 days at ≈ 100% rh (B, D). Significant ($p \leq 0.05$) differences between 2 adjacent sampling times are denoted by the placing of a * beside one histogram and above the other.

the dried seed (Fig.20). U_1 declined significantly between 20 and 23 DAFB in both dried fresh and stored seed and again declined significantly between 25 and 27 DAFB in the dried fresh seed. Although this decline in the level of U_1 with age would precede the development of the ability to withstand desiccation, storage did not affect the U_1 levels (Table 15). U_2 also declined with age, however a significant decline was not found until 30 DAFB in both dried fresh and stored seeds. At 25 DAFB the level of U_2 in the dried stored seed was significantly lower than in the dried stored seed. U_3 on the other hand, again declined significantly between 20 and 23 DAFB in the dried fresh seed and thereafter was found in relatively constant levels. In the dried stored seed although there was a tendency for the U_3 levels to decrease, there were no significant changes. The level of U_3 found at 20 DAFB was significantly lower in the dried stored seed than in the dried fresh seed (Table 15).

Only one phospholipid - U_5 was found in the second group. This was characterised by a significant increase in levels between 23 and 25 DAFB in both the dried fresh and stored seed with a further increase in level between 25 and 27 DAFB in the dried stored seed (Fig.20). Rather contradictorally, there was a significantly lower level of U_5 found in the dried stored seed at 25 DAFB.

Thus with both age and storage in dried seed, which again coincides with an improvement in embryo condition there was a general tendency for an increase in the levels of total phospholipids and PC. Levels of PI, PG and U_5 increased with age but appeared to remain unaffected by storage. However, the levels of U_1, U_2, U_3, U_6 and PA tended to decrease with an improvement in

embryo condition, i.e. they decreased both with age and storage. Again PE and phosphorus at the origin decreased with age but not with storage.

Thus in summary, with improved retention of solutes and the development of the ability to withstand desiccation there was a tendency for the levels of PI, PC (hence the total), PG and U_5 to increase while the levels of PE, PA, phosphorus at the origin, U_6, U_1, U_2 and U_3 decreased. High humidity storage, which improves solute retention and seed viability resulted in an increase in PC. The levels of U_6 and PA tended to decrease with both age and storage.

One of the most striking differences between succulent and dried seeds was that the levels of total phospholipids, PI, PC, PG and PA were markedly depressed in the dried seed but the levels of phosphorus at the origin, PE, U_4 and U_6 found in the dried seed were similar to the levels found in the succulent seed. Drying produces another difference between succulent and dried seed in the appearance of four unidentified phospholipids. Of these, three declined with improved embryo retention and viability (U_1, U_2 and U_3) while U_5 increased. On drying the levels of PA and PE both decrease with age and with storage.

Section 4 - The influence of the testa and maturity on the germination of succulent seeds

So far, in this thesis changes in the germination ability of seeds during development has focussed attention on the viability and condition of seeds after desiccation. Little work has been done on the germination ability of seeds before desiccation, indeed, in the Introduction the literature was found to be somewhat contradictory with respect to the germination ability of succulent seeds and the role of the testa.

The effect of the presence of the testa on the germination of succulent seeds was initially tested by subjecting three replicates of 20 succulent seeds with their testae and three replicates of 20 succulent seeds without their testae, to a germination test in sand. Germination of seed, as seen by the production of a plumule above the surface of the sand was found at 25 DAFB (Fig.21A). The germination capacity of seeds both with and without their testae generally increased with age. However, from 25 DAFB to 43 DAFB the number of seeds that germinated without their testae was significantly greater than those which germinated with their testae (Fig. 21A). By 50 DAFB there was no significant difference between the germination ability of succulent seeds with or without their testae.

The use of the percentage germination gives a good measure of the germination capacity of the seeds but it obscures any differences there might be in the rate of germination. To try and express differences in germination rate Kotowski's 'coefficient of velocity of germination' was used. This measured the rate at which viable seeds germinate; the larger the coefficient the more rapid the

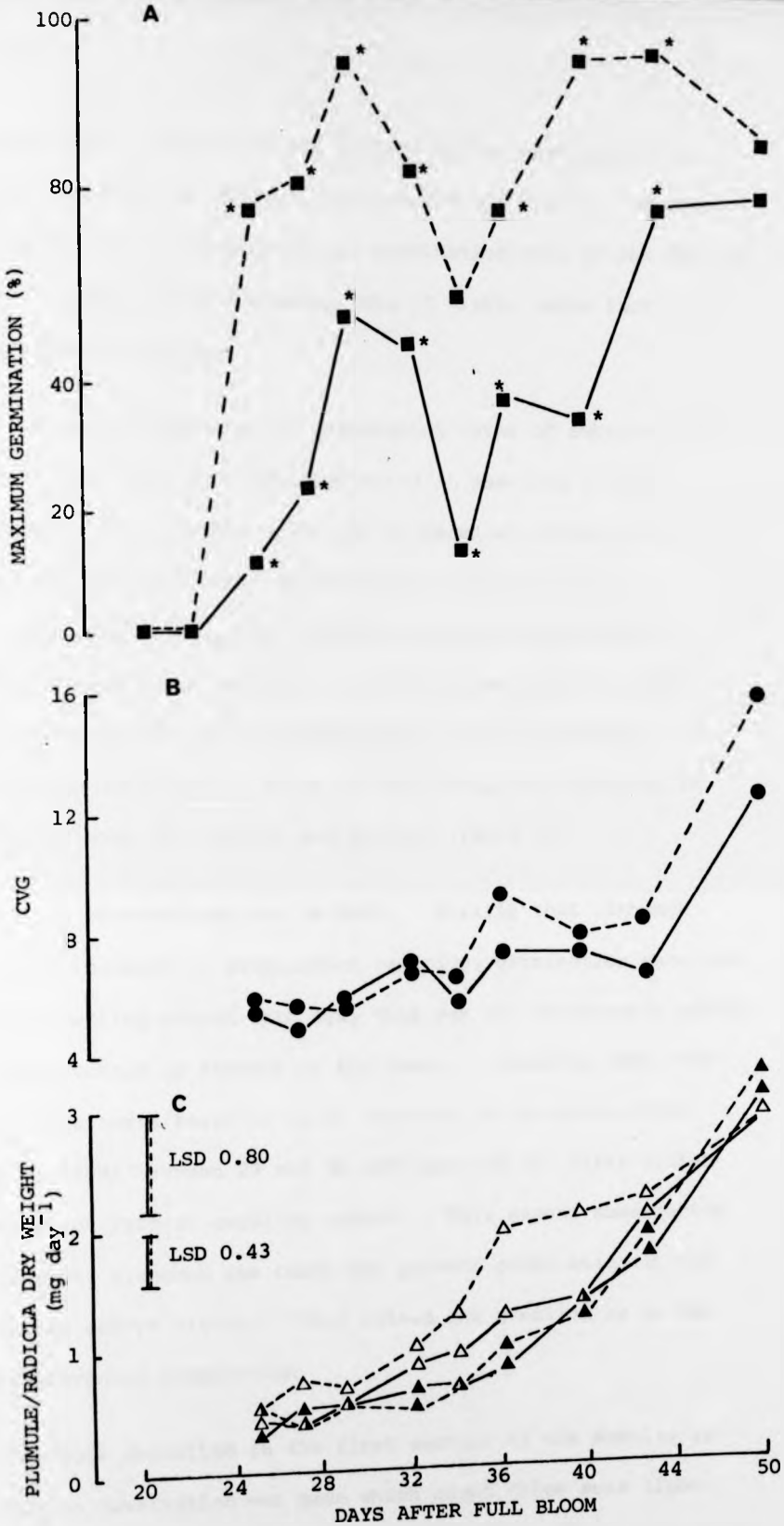


FIG.21 Maximum germination (■), the co-efficient of velocity of germination CVG (●) and radicle (Δ) and plumule (▲) dry weights produced by immature seeds set to germinate with (——) or without (---) their testae

germination rate. When this was applied to the germination of succulent seeds with or without their testae the presence or absence of the testa had no influence on the germination rate of the embryos (Fig.21B). Again the germination rate of viable seeds increased with increasing maturity.

This lack of difference in germination rates of embryos with or without their testa was also reflected in seedling growth. The seedlings produced from seeds set to germinate without their testae showed no significant differences in either plumule or radical dry weight (Fig.21C) from those produced from seeds set to germinate with their testae. As before, the seedling growth measured by dry weight of the radicle and plumule, increased with increasing age (Fig.21C). These patterns were also reflected in the length of both the radicle and plumule (Table 16).

Thus two observations can be made. Firstly that although there was an increase in germination capacity, germination rate and subsequent seedling growth with age, this was not necessarily affected by the presence or absence of the testa. Secondly, that the absence of the testa resulted in an increase in the germination capacity of seeds between 23 and 30 DAFB but did not alter either the germination rate or seedling growth. This second observation suggested that, although the testa may prevent germination it did not influence embryo vigour. This raised the question as to how the testa prevented germination.

In the work described in the first section of the Results in this thesis an observation was made which might throw some light on this question. When the effect of adding extra respiratory

TABLE 16

Increase in Radicle and Plumule lengths (mm day^{-1}) produced by immature seeds set to germinate with or without their testae

Days After Full Bloom		25	27	29	32	34	36	39	43	50	LSD
Radicle	+t	0.07	0.21	0.29	0.31	0.38	0.49	0.62	0.68	1.18	0.17
Length (mm day^{-1})	-t	0.16	0.18	0.24	0.31	0.25	0.50	0.60	0.71	1.17	
Plumule	+t	0.05	0.20	0.26	0.28	0.27	0.39	0.49	0.57	0.96	0.22
Length (mm day^{-1})	-t	0.20	0.21	0.30	0.20	0.20	0.38	0.59	0.88	1.26	

Seeds were set to germinate with their testa present (+t) or after their testa was removed (-t) and plumules and radicles harvested 7 days after germination was first observed. Older seeds germinated more rapidly than younger seeds. To allow for these differences in germination rates subsequent growth measurements were expressed as mm day^{-1}.

substrate to developing seeds was being investigated, removal of the testa resulted in an increase in the respiration rate of the embryo; this was more marked in older seeds. This led to the suggestion that the testa may be a barrier to gaseous diffusion, thus the difference in germination capacity observed might be explained in terms of the immature embryos being unable to respire sufficiently within the testa when they were set to germinate.

When the respiration rate of seeds with and without their testae was measured (five replicates of five seeds) it was found that after 29 DAFB the respiration rate of the embryos (i.e. seeds without their testae) was greater than that of the whole seed (Fig. 22). Thus only after 29 DAFB was the testa apparently restricting the respiration rate of the embryo. Before 29 DAFB the influence of the testa on embryo respiration was not so apparent. Until 29 DAFB the respiration rate of the embryos was less than the respiration rate of the whole seed; the differences in rates being attributed to the respiration rate of the testae itself. Between 25 and 29 DAFB the respiratory levels of both embryo and seed are sufficiently close that it is likely that embryo respiration is restricted by competition with testa respiration. However, before 25 DAFB, when no succulent seeds were able to germinate the respiration rate of the embryos is extremely low and restriction of their respiration by the testa is unlikely. Thus, although after 29 DAFB removal of the testa resulted in an increase in the number of seeds germinating, possibly by allowing the embryo to respire at its maximum rate, it did not explain the fact that once germinated embryo vigour and seedling vigour were the same whether the seed was set to germinate with or without its testa. This may be partially

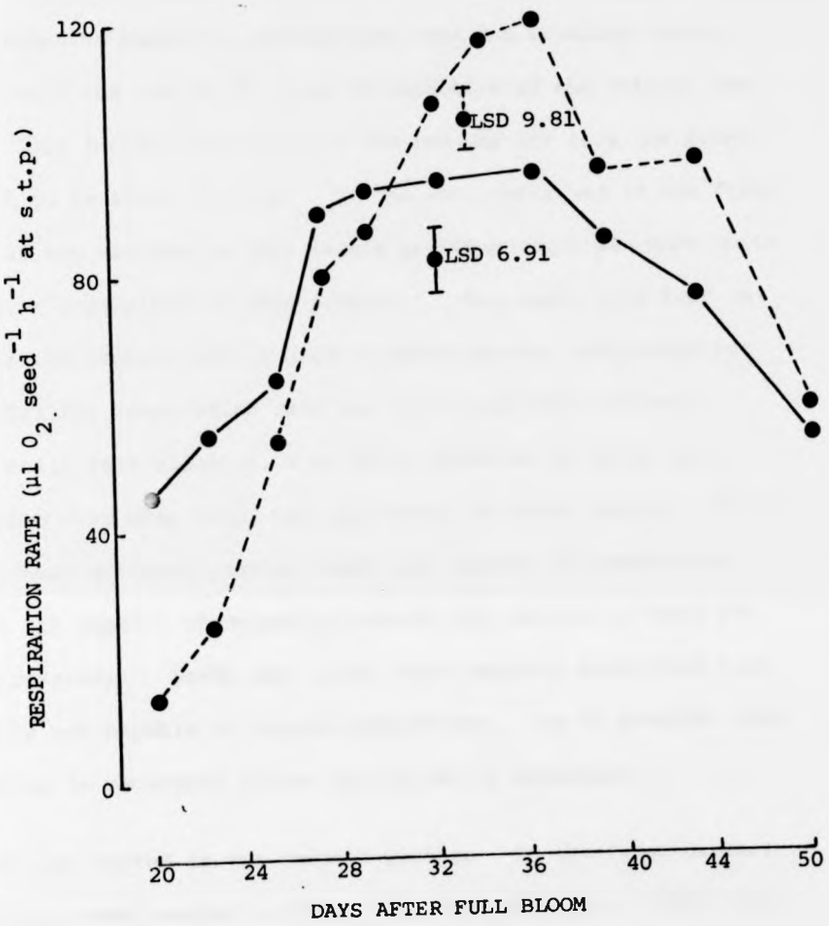


FIG.22 The respiration rate (µl O_2 $seed^{-1}$ h^{-1} at s.t.p.) of 5 replicates of 5 seeds with (——) or without their testae (---) during normal development

explained using the observation that the testa, throughout the period studied was thick and fleshy which would present a considerable physical barrier to a growing radically especially if the vigour of the seed was itself low.

Germination capacity, germination rate and seedling vigour improved with the age of the seed irrespective of the role of the testa. This focuses attention on the reasons for this low growth potential in immature embryos. In the work described in the first section of the results in this thesis an observation was made which might throw some light on this problem. When seeds were kept in high humidity storage with a high moisture content maintained but with a falling respiration rate and a falling sugar content, starch levels rose slightly, i.e. there appeared to be no starch degradation even when sugar was apparently in short supply. It is possible that although younger seeds are capable of germination they are not capable of degrading starch thus utilizing their own storage reserves. Seeds kept under high humidity conditions were apparently not capable of starch degradation. Is it possible that some drying is necessary before starch can be degraded?

This was tested in two harvest series. In the first harvest series seeds were sampled and kept for three days under 100% rh and 50% rh conditions (Fig.23A). In the second harvest series, after sampling, seeds were kept for three days under 100% rh and 40% rh conditions (Fig.23B). Measurements of moisture content were obtained from five replicates of five seeds at each time of sampling and after three days in each storage condition. The moisture content of the seeds sampled initially increased throughout the period studied (Fig.23) and the moisture content of the seeds kept

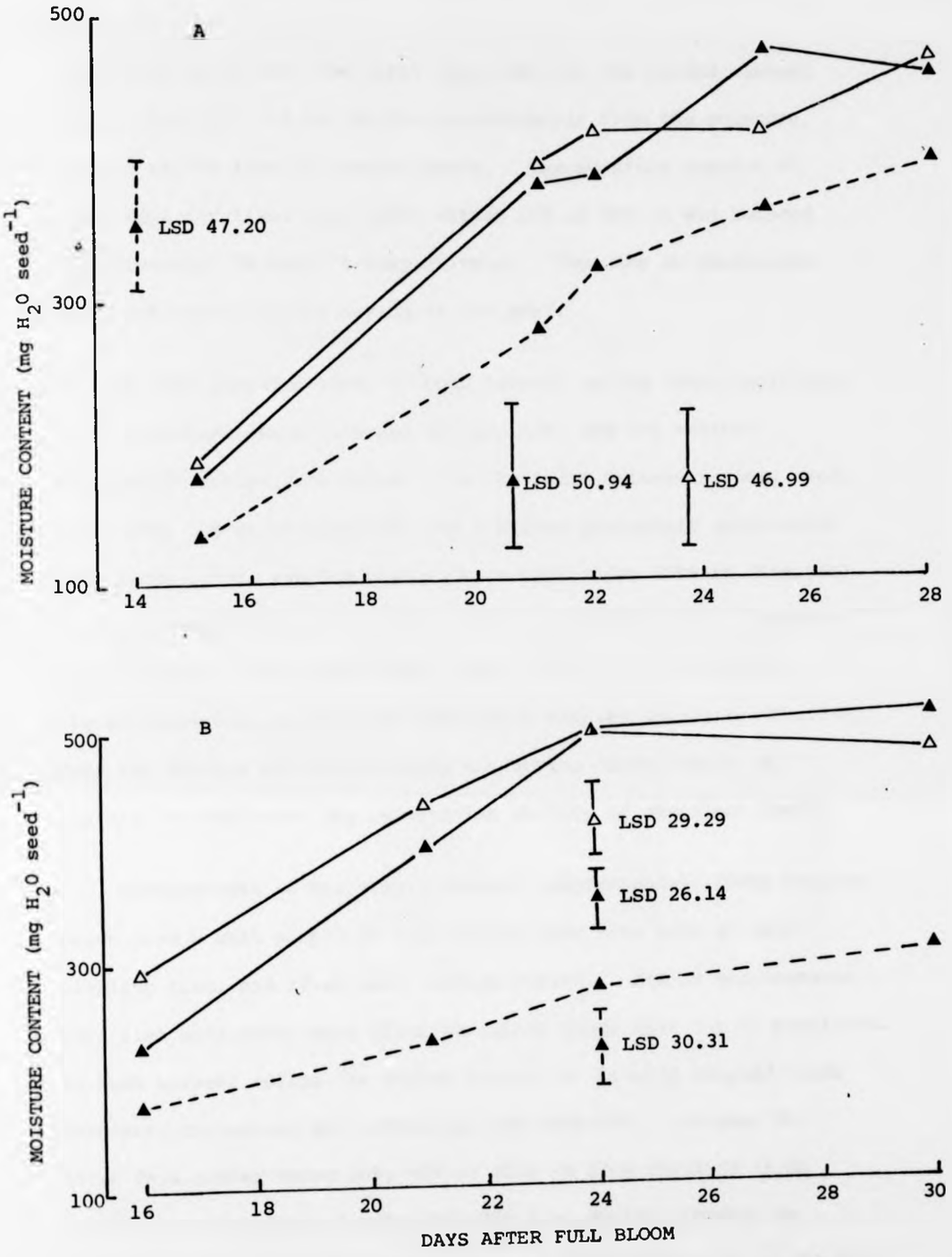


FIG.23 The moisture content (mg $seed^{-1}$) of 5 replicates of 5 seeds sampled freshly (Δ A and B), stored for 3 days under 100% rh (—▲— A and B) or stored for 3 days under 50% rh (--▲-- A) or 40% rh (--▲-- B) in two separate harvest series

under 100% rh in both the first (Fig.23A) and the second harvest series (Fig.23B) did not differ significantly from the moisture content of the freshly sampled seeds. The moisture content of seeds kept for three days under either 50% or 40% rh was reduced approximately 5% and 10% respectively. Thus the rh conditions used, achieved a little drying of the seed.

At each sampling time, in both harvest series three replicates of 20 succulent seeds were set to germinate and the maximum germination achieved recorded. In the first harvest series, seeds kept under 50% rh consistently had a higher percentage germination than seeds either sampled initially or kept under 100% rh (Fig.24A). In the second harvest series this was not quite so clear, although seeds kept for three days either under 40% or 100% rh showed a higher percentage germination than seeds sampled initially (Fig.24B). Thus the storage and particularly the drying conditions used, appeared to influence the germination ability of succulent seeds.

Measurements of the starch content (approximately 200mg samples drawn from a bulk sample of five seeds) were also made at each sampling time, and after each storage period. Starch measurements were also made seven days after succulent seeds were set to germinate. In both harvest series the starch content of freshly sampled seeds increased throughout the period studied (Fig.25). Storage for three days either under 50%, 40% or 100% rh also resulted in an increase in the starch levels (Fig.25) i.e. during storage, as before there was no starch degradation. This pattern of an increase in starch was also found in the seeds sampled initially at each sampling time seven days after they were set to germinate (Fig.25),

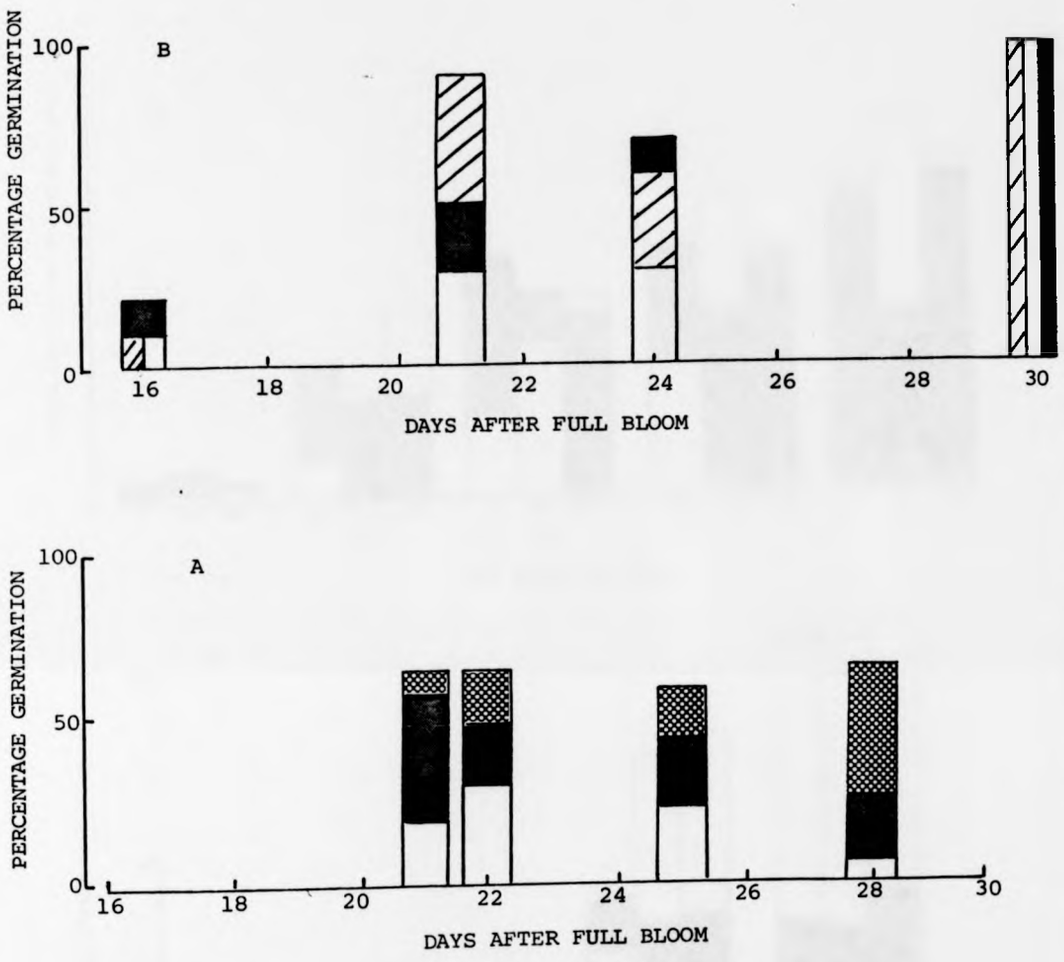
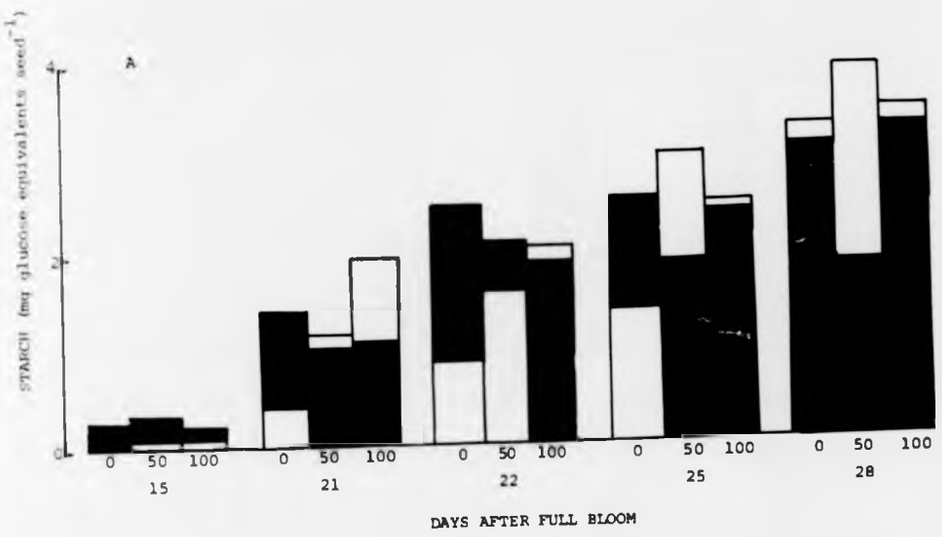


FIG.24 Percentage germination (3 replicates of 20 seeds) of seeds set to germinate immediately after sampling (□) or after being subjected to 3 days at 100% (■), 50% (▩ A) or 40% (▨ B) rh



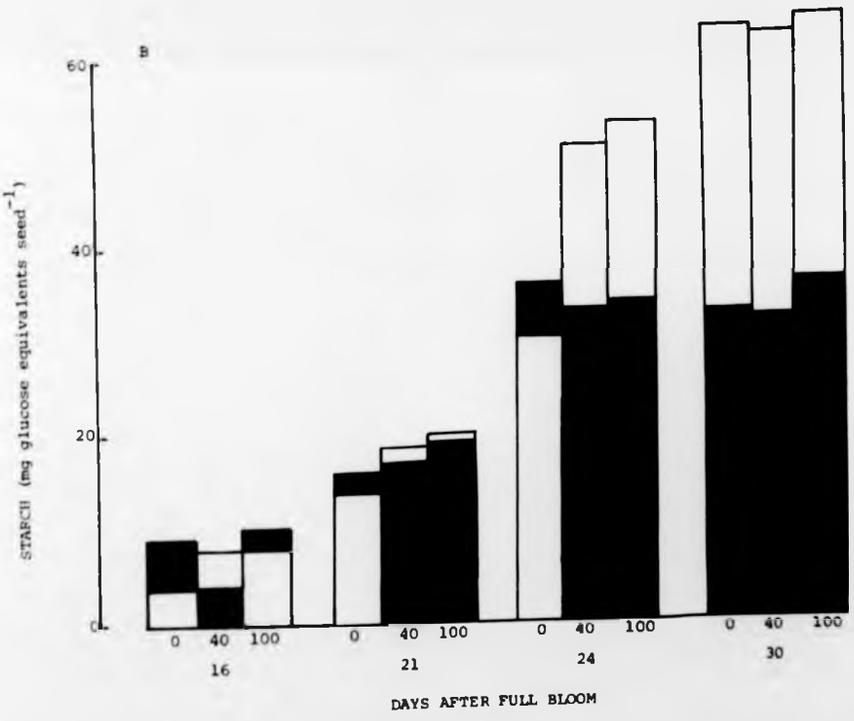


FIG.25 Changes in starch content (bulk sample of 5 seeds) in harvest series 1 (A) and in harvest series 2 (B) in seed sampled freshly (O) or stored for 3 days under either 100%, 50% or 40% rh. □ - seeds sampled initially ■-seeds sampled 7 days after being set to germinate.

with the exception of the oldest seeds in both harvest series. Seeds subjected to some drying under either 50% or 40% rh conditions showed a decrease in the starch levels found seven days after the seeds were set to germinate (Fig.25). Apparently, holding seeds under the drying conditions used produced degradation of starch but a similar pattern of starch degradation was also found in seeds which had been kept for three days under 100% rh. Therefore, partial drying of the seeds does not satisfactorily explain the ability of succulent seeds to degrade starch seven days after they are set to germinate. The observation that seeds of different ages set to germinate immediately after being removed from the plant did not have the ability to degrade starch seven days after they were set to germinate is of interest. It may well be that the only difference between seeds held for three days and seeds set to germinate immediately is that seeds held for three days functioned in effect for ten days before starch levels were measured as compared to seven days for seeds set to germinate immediately. Thus it is possible that the ability of succulent seeds to degrade starch was a function of time with the rate of reaction being so slow in immature seeds that the vigour of the seed is affected.

DISCUSSION

One of the most important steps in the evolution of the Angiosperms has been the development of the seed habit, characterised in temperate annual species, by the production of a desiccated seed, with a low level of physiological activity, capable of withstanding unfavourable conditions and containing sufficient food reserves for the establishment of a seedling once conditions become favourable. This thesis, using pea seeds as experimental materials, has been concerned with two aspects of seed development. Firstly, and primarily, an attempt has been made to determine what physiological changes are necessary before the development of the ability to withstand desiccation, including changes in the phospholipid constituents of cell membranes, can take place and using this information, some attempt has been made to describe the characteristics of seeds that are able to withstand desiccation. Secondly, the development of the ability to germinate by seeds taken directly from the pod has been examined and some of the processes which might be involved in preventing precocious germination investigated.

Physiological changes and tolerance to desiccation

Most of the work in this investigation has been an attempt to determine what changes are crucial for the development of the ability to withstand desiccation by seeds. These changes will be discussed in relation to other work on physiological and ultrastructural changes in developing seeds.

Previous work has suggested that a decline in moisture content resulted in a reduction in the physiological activity of the seed

and that this in turn brought about the development of the ability to withstand rapid desiccation (Matthews, 1973a). The findings in this thesis have confirmed that in pea seeds the development of the ability to withstand rapid drying to low moisture contents is preceded by a fall in respiration rate, but they do not support the idea that a fall in respiration rate is triggered off by a fall in the moisture content of the developing seed. The fall in the respiration rate of the seed was preceded by a fall in the ethanol soluble sugars. This fall in sugars also occurred prior to a fall in the moisture content of the seed which agrees with the findings of McKee et al., (1955), Danielson (1956) and Robertson, Highkin, Smydzuk and Went (1962). Although this change was not apparent in the work of Bisson and Jones (1932) and Bain and Mercer (1966) it may have occurred but was undetected because the sampling times used missed the peaks of moisture and sugar content.

Thus it can be suggested that a fall in the amount of sugar available as respiratory substrate is more critical in bringing about a fall in the level of physiological activity of the seeds, as measured by O_2 uptake, and the subsequent development of the ability to withstand rapid desiccation than has previously been supposed. Several pieces of evidence support this hypothesis. When the moisture content of seeds was maintained at a high level, a decline in the sugar content and in the physiological activity of seeds was seen. No such changes were found in seeds of the same age that had been left attached to the parent plant. A fall in respiration rate under conditions where moisture was high has also been shown for soybeans by Howell, Collins and Sedgwick (1959) who suggested that this fall was associated with a partial depletion of respiratory substrate. When extra respiratory substrate in the form of

sucrose was supplied to pea embryos which had previously been kept under high humidity conditions and had both a falling sugar content and a falling respiration rate the 0_2 uptake of the embryo was significantly increased.

Further, when attempts were made to limit the supply of assimilates from the parent plant to the developing seed by defoliation and dark treatments the sugar content of the seeds was reduced and this again preceded a fall in the respiration rate of the seed. In all these cases the falling sugar levels and respiration rate were accompanied by improved embryo condition after rapid drying as measured by the leaching of electrolytes from seeds over a 24h period. This improvement in embryo condition was generally although not always accompanied by an increase in the viability of seeds indicated by tetrazolium staining of the embryo axis or by germination tests in sand.

This hypothesis, that the development of the ability of seeds to withstand desiccation is dependent on a fall in physiological activity brought about by a fall in sugar content, was further supported by work carried out using larger samples of seeds with a wider variation in age grown both in the glasshouse and in the field. The successful use of high humidity treatments to reduce sugar content and increase the number of seeds able to withstand desiccation provides not only support for the hypothesis postulated but also suggests a practical application of high humidity storage.

When peas are grown for seed they are harvested in the late summer - early autumn after the seeds have dried out. The likely occurrence of wet weather around the time of harvest has limited

the production of seed peas in the United Kingdom, with about half of the seed peas used in the United Kingdom being grown in countries with dry, hot summers such as Hungary, U.S.A., Canada and New Zealand (Charles Sharpe & Co. Ltd., pers. comm.). Wet weather after seeds have dehydrated has several effects. The seeds can become rehydrated, as has been shown for soybeans (Howell et al. 1959) and for peas (Matthews, 1973b) which reduces their final percentage viability (Flentje, 1964; Matthews 1973b). Furthermore, due to high humidities it is often difficult to dry the crop to a sufficiently low moisture content (Davies, 1977). This problem of insufficient drying is accentuated by the tendency of pea plants to lodge, resulting in seeds developing in a closed humid environment (Davies, 1977) making normal drying more difficult. The humid conditions also results in the development of seed borne micro-organisms both pathogens such as Ascochyta pisi and Mycosphaerella pinoides (Jones, 1927) and saprophytes such as Rhizopos sp., Botrytis sp. and Aspergillus sp. (Flentje, 1964; Wallen and Skolka, 1950) which reduce the emergence of peas (Flentje, 1964; Matthews, 1973b). Some of these problems could be avoided if seeds could be harvested while the moisture content was still high and treated in such a way that viable seeds were still produced. The premature development of the ability to withstand desiccation could be brought about by holding seed in high humidity storage directly after harvesting, provided that seeds were harvested around the peak period in physiological activity. This proviso is necessary in that if seeds are too immature drying will result in death anyway and if seeds have dried too much, high humidity storage may cause the same type of rehydration damage that has been noticed in field grown material subjected to rain before harvest. If the process

involved in enabling the seeds to withstand desiccation have progressed beyond a certain point, as would be found during normal drying, high humidity conditions would be detrimental by rehydrating systems which may already have been inactivated. Thus, for high humidity storage to be used on a practical scale a simple method of closely assessing the state of physiological activity of the crop must be found. A major problem in the harvesting of seeds at a high moisture content and then subjecting them to high humidity treatments, is the danger of fungal infection. At present, current agronomic advice is to slowly dry seeds after harvest (Kreyger, 1963), it is likely that a combination of holding seeds in high humidities then subsequently drying them relatively slowly would have the desired effect of producing viable seeds.

Part of the evidence for the influence of sugar levels on the level of respiratory activity of the developing seed was obtained by reducing the supply of assimilates from the plant to the seed. When defoliated plants were placed in the dark, the sugar content and the respiration rate of the developing seeds declined while embryo condition after desiccation improved although viable seeds were not produced.

These changes occurred despite the continued increase in dry weight which was accompanied by an increase in starch. Thus, although the sugar content of the seed declined starch synthesis continued to increase. It is possible that the synthesis of starch is an over-riding process in seed development such that even when the supply of respiratory substrates such as sugars are reduced starch synthesis continues to increase which would in turn further reduce the amount of sugars available for respiration.

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Not all attempts to manipulate the assimilate supply from the parent plant to the seed were successful. Simply placing pea plants with developing seeds in the dark did not result in a reduction in physiological activity. Seeds continued to show the same pattern of changes in respiration rate in both the control and dark treated plants. The intended effect of the dark treatment, to reduce assimilates from the plant to the seeds, was apparently not achieved since in plants remaining in the dark for ten days the dry weight continued to increase although after two days in the dark it increased more slowly than it did in control plants. Thus although there appeared to be a slowing down in dry weight increase in the dark treated plants and by implication a reduction in the amount of assimilates available to act as substrates for synthetic reactions such as starch or protein synthesis, this reduction was not sufficient to affect the level of physiological activity of the seeds. This apparent lack of a substantial reduction in assimilate supply even after ten days in the dark is noteworthy in view of the heavy dependence of fruit filling on current photosynthesis (Pate 1975). In P. sativum the carpel fixes substantial amounts of carbon which is subsequently exported to the developing ovules (Lovell and Lovell 1970) while the leaf can export 90% of its photosynthates to the carpel and ovules (Linck and Sudia, 1962). Developing ovules not only receive assimilates from the leaf and carpel on its own node but they also receive them from other nodes especially vegetative nodes (Harvey 1973). In P. arvense Flinn and Pate (1970) have shown that the seed obtains about 85% of its total carbon requirements from photosynthates from stipules, leaves and pods with the rest being supplied by mobilization of dry matter that had previously been fixed. The mobilization of dry matter has not been observed

in P. sativum directly before, but the fact that plants held in the dark for ten days still showed an increase in dry weight would strongly support the idea that mobilization of previously assimilated dry matter is occurring in plants of P. sativum which are not photosynthesising.

During the normal course of development, before the seed can withstand desiccation, the level of physiological activity must drop and it is postulated that this is initiated by a reduction in the level of sugars. Throughout this thesis, physiological activity has been measured in terms of O_2 uptake but this assumes that respiratory activity is a good indication of physiological activity and that the dominant respiratory pathway in use in developing seeds is through glycolysis, the TCA cycle and a terminal oxidase system. This is not necessarily the case. It may be that there are changes in respiratory metabolism not measurable by O_2 uptake that take place during seed development. In an attempt to investigate this problem respiratory activity was measured using both O_2 uptake and CO_2 output. The pattern of changes in O_2 uptake roughly paralleled the pattern of change in CO_2 output, the main difference being that during normal development the CO_2 level fell more rapidly after a peak in respiratory activity than did the O_2 level. This more rapid fall in CO_2 output, resulting in a decreasing RQ could either be caused by some of the CO_2 becoming fixed as would be the case if a photosynthetic system became active or if there was a switch in the type of respiratory substrate used. The possibility of the activity of a photosynthetic system especially around the peak in respiratory activity was tested by measurements of O_2 uptake and CO_2 output being made in both the light and in the dark. There were no significant

differences found between CO_2 output levels in the light or in the dark suggesting that there was no light fixation of CO_2. This was further supported by the similarity found in the RQ of seeds of the same age in both the light and dark.

The reduction in the value of the RQ which was found with increased age could have been brought about by a change in the type of substrate being respired (Beevers, 1961). In the very young seeds which still have some liquid endosperm present the RQ is 1.0, indicating that at a time when seeds contain high levels of sugars that the embryo is predominantly respiring carbohydrates. During development, while the overall respiration rate is still rising the RQ decreases to around 0.7 suggesting that the seeds are respiring some form of fats. After the peak in respiratory activity i.e. when the respiration rate begins to fall, the RQ falls even further to between 0.5 and 0.6. By the time respiratory activity and moisture content are at a low level and starch content is increasing rapidly the RQ falls to between 0.2 and 0.3.

An alternative explanation of this fall in RQ is that some of the CO_2 evolved in respiration is being fixed in a light independent process. It has been suggested that RQ levels below 0.5 in plant tissues may indicate that some of the CO_2 was being fixed (Beevers 1961, Baron, 1967) over the time period studies there was no evidence for the photosynthetic fixation of CO_2 in developing pea seeds. The possibility of some dark fixation of CO_2 cannot be ruled out especially in view of the observation of Hedley, Harvey and Keely (1975) that during the latter stages of pea seed development the level of P.E.P. carboxylase in the cotyledons, an enzyme involved in dark fixation, increased. They envisaged this being the basis of a dark

fixation system activated during early germination of the seed. It may be that such a system could also operate in older pea seed which would partly explain the low RQ values found in the oldest seed.

Even if there is dark fixation of CO_2 during at least the later stages of seed development there still appears to be a change in the type of respiratory substrate utilized during seed development. This produces a problem in that early in development, prior to a peak in physiological activity seeds appear (on the basis of their RQ's) to be respiring fat but at the same time it has been postulated that a reduction in physiological activity is brought about by a reduction in sugars available for respiration. This could be explained by seeds respiring fats while sucrose is unavailable due to its preferential use in maintaining the osmotic potential of the cell (Bain and Mercer, 1966) and in starch synthesis. As fats become exhausted in seeds separated from the parent plant sugars will become available for respiration and supplies of sucrose in turn become exhausted and addition of sucrose at this stage would result in an increase in respiration rate.

Thus during seed development there may be changes in the type of substrate being respired and there is the possible implication of a dark fixation pathway late in seed development. However, the original problem as to whether there is more than one dominant respiratory pathway in use during development has still to be discussed. It has been postulated that there is an 'alternate respiratory pathway', insensitive to cyanide found during the first 5h in germinating pea seeds. Since it is not inhibited by cyanide it must involve a terminal oxidase system other than that involving the cytochromes and it has been suggested that this 'alternate' oxidase may be a

flavoprotein located on the outer mitochondrial membrane the action of which is inhibited by hydroxamic acids (Wilson and Bonner, 1971). The functioning of this type of respiratory pathway very early in germination may indicate that it is present in the developing seed prior to drying. In the present work the use of SHAM in concentrations which inhibited 'alternate respiration' in germinating peas (Yentur and Leopold, 1976) had no effect on the respiration rate of developing seeds whether the respiration rate was measured using either 0_2 uptake or CO_2 output. However, when the KCN was used in the same concentrations, 0_2 uptake was severely inhibited. Therefore, in seeds respiring over the time period studied, namely from phase 2 (Bain and Mercer, 1966) which is characterised by cellular expansion until the middle of phase 4, characterised by severe dehydration of the embryo, respiration was via a terminal oxidase containing either copper or iron. It is possible to say this since the action of the terminal oxidase is blocked by cyanide forming stable complexes with these metals. No attempt was made to specifically identify the terminal oxidase involved but cytochrome oxidase systems have been shown to be present in developing embryos and in young pea seedlings (Goddard and Meeuse, 1950) and more significantly Kollöffel (1970a, b) has shown that cytochrome oxidase activity declined during the maturation of pea cotyledons.

The effect of KCN on CO_2 output during development is of interest in that after the peak in physiological activity the presence of KCN resulted in an increase in CO_2 production, thus with the blockage of the TCA-terminal oxidase cycle anaerobic repiration appeared to be stimulated. Two possible explanations can be put forward for this in that glycolysis may be stimulated in a Pasteur

effect or, it is possible that there is increased activity of the Pentose Phosphate Pathway especially in a situation where biosynthetic reactions were particularly active. The lack of an increase in CO_2 production in the presence of KCN in seeds prior to the peak in physiological activity is surprising.

Thus during development there appear to be numerous changes occurring in respiratory systems. Firstly, there appears to be a change in respiratory substrates utilized by the seed. Secondly, the activity of pathways such as the Pentose Phosphate Pathway are implicated especially in older seed and thirdly, it is also possible that a dark fixation pathway is present in the oldest seeds studied. Of these three factors all are probable and it is not possible to conclusively separate out these factors on the basis of the work carried out in this thesis. The final and predominant change occurring during development is the reduction in activity of the TCA-terminal oxidase system seen by a reduction in both 0_2 and CO_2 levels. Kolloffel (1970a, b) in peas and Lado (1965) in castor beans showed that there was not only a reduction in cytochrome oxidase activity with age but that as seeds developed there was a deficiency of cytochrome c. Kolloffel (1970a, b) concluded that during the maturation of pea seeds there is a gradual reduction in the efficiency of respiratory control and phosphorylation activity. The findings here would support this idea namely, that it is possible that the parts of respiration showing greatest reduction in activity during development were the TCA-terminal oxidase system. Thus 0_2 uptake would appear to be a valid measure of physiological activity which clears the way for some discussion of the relationship between the changes in physiological activity and changes on an ultrastructural level.

Work has been done on the ultrastructural changes occurring during the development of pea seeds (Bain and Mercer, 1966), lima beans (Klein abd Pollock, 1968) dwarf beans (Opik, 1968) and soybeans (Bils and Howell, 1963). These studies basically agree that the final stages of maturation of leguminous seeds are characterised by the disappearance of the golgi, the mitochondria becoming less distinct, the polysomes becoming degraded and the ER becoming reduced. Despite this it is difficult to pinpoint the time at which these changes occur during the course of development. Most of these studies have used changes in weight and moisture content as a measure of the state of maturity of the developing seed. From the work in this thesis the respiration rate (measuring physiological maturity) and moisture content of the seed show similar patterns of changes such that a fall in respiration rate is reflected by a fall in moisture content. Therefore, if a peak in moisture content is taken as an indication of the peak in physiological activity then this coincides with phase 4 of development described by Bain and Mercer (1966), by the final phase described by Klein and Pollock (1968) and by phase 4 described by Bils and Howell (1963). If the level of physiological activity is critical as to whether the seed can withstand desiccation or not then the most logical connection between ultrastructural and physiological events could be considered to be the mitochondrial system and respiratory activity. Using moisture content as an indication of physiological activity, at around the time of peak physiological activity when the mitochondria begin to become more difficult to resolve microscopically (Bain and Mercer, 1966; Klein and Pollock, 1968), the activity of the mitochondrial fraction itself decreases (Bils and Howell, 1963; Lado, 1965;

Kollöffel, 1970b). It is also at this time that there is a reduction in respiration rate whether measured by O_2 uptake or CO_2 output. This reduction in respiration rate is only partially reversible in that if extra respiratory substrate is added the rate will increase again but never return to its original level. In the discussion of the respiratory pathways involved it was suggested that the TCA-terminal oxidase system was more seriously affected by the drop in respiratory activity than was glycolysos and it was not until this time that the ability to withstand desiccation develops. It is possible, since the enzymes of the TCA cycle and electron transfer chain are in the mitochondria while the enzymes of glycolysis are located in the cytoplasm that the observed changes in mitochondrial structure can now be given some physiological significance. Thus, the development of the ability to withstand desiccation appears to be associated with a reduction in the activity of the TCA-terminal oxidase system which corresponds with observed changes in the internal appearance of the mitochondria.

Therefore a reduction in respiratory activity is either caused by or is the result of a reduction in the efficiency of the mitochondria however it has already been shown that a reduction in the sugar levels results in a reduction in respiration rate and the question arises as to how theoretically these events can be interrelated. It may well be that physiological changes which occur during seed development may have their connection with ultrastructural changes much earlier in development. Bain and Mercer (1966) suggested that the fall in sugar levels could be accounted for by the rise in starch levels, but the levels of starch synthesis are not dependent on the sugar supply (Danielson, 1956; Robertson et

al, 1962) rather, they are dependent on the level of starch phosphorylase activity (Turner et al 1957; Robertson et al., 1962). It is also of interest that there is an increase in protein nitrogen levels in cotyledon cells (McKee et al., 1955) when there is a general expansion in cell organells (Bain and Mercer, 1966; Klein and Pollock, 1968). It is feasible that the initial fall in respiration rate apparently due to a shortage of sugars was the result of starch synthesis increasing to such a rate, that starch synthesis and respiration were in direct competition for available carbohydrates with starch synthesis "winning", resulting in a reduction in respiration. This reduction in respiration rate could have been either facilitated or caused by a reduction in mitochondrial activity. Thus it is possible that changes in physiology and changes in ultrastructure may be parellel changes with intermittent interlinking events such that for example, an increase in cell organelles and membranes which are characteristic of the young developing seed lead to an increase in the synthetic activity of the cell which could in turn result in a reduced respiration rate. A reduced respiration rate would in turn result in less energy being available for cellular processes. At the same time an apparently 'condensing' ultrastructural anatomy as seen in phase 4, which has amongst other changes, an apparent reduction in mitochondrial efficiency, possibly influenced by the falling respiratory activity, would in turn have an influence in reducing the efficiency of the TCA cycle. If this line of reasoning is possible there are still many gaps which need filling, e.g. what is the nature of the connection between an expanding cytoplasm, ultrastructurally speaking, and increased synthetic activity; what is the effect of a reduction in energy supply on the subsequent physiological and

ultrastructural changes in pea seed and most importantly what is cause and what is effect?

This section of the discussion is primarily concerned with what changes occur in the phospholipid content of membranes during seed development and with how these changes can be related to the ability of seeds to withstand desiccation. These questions arose from the observation that the embryo condition as measured by the electrical conductivity of seed leachates improved with the age of the seed i.e. conductivity decreased. This has also been found in developing pea seeds by Matthews (1973a) for two harvest series using cv. Kelvedon Wonder and by Bedford and Matthews (1976) in one harvest series using cv. Jade when conductivity was expressed on a g^{-1} d. wt. basis. However, before the possible implication of this on a subcellular basis can be considered, closer scrutiny must be made of the original observations of decreased leaching and of their interpretation.

One question that arises from these observations is whether this decrease in conductivity is a real measure of an increase in the ability of seeds to retain solutes or whether it could have arisen from the way the results were expressed. In this thesis the 24h conductivity reading has been expressed g^{-1} d.wt. so making allowance for the air dry weight of the seed and when expressed in this way the 24h conductivity decreases throughout (Fig.3A). However, during this period seed dry weight is increasing and it may be that the fall in conductivity g^{-1} d.wt. resulted from an increase in dry weight. When the same conductivities are expressed on a $seed^{-1}$ basis there is first an initial rise but the level subsequently falls (Fig.26) despite a continued increase in dry weight. This

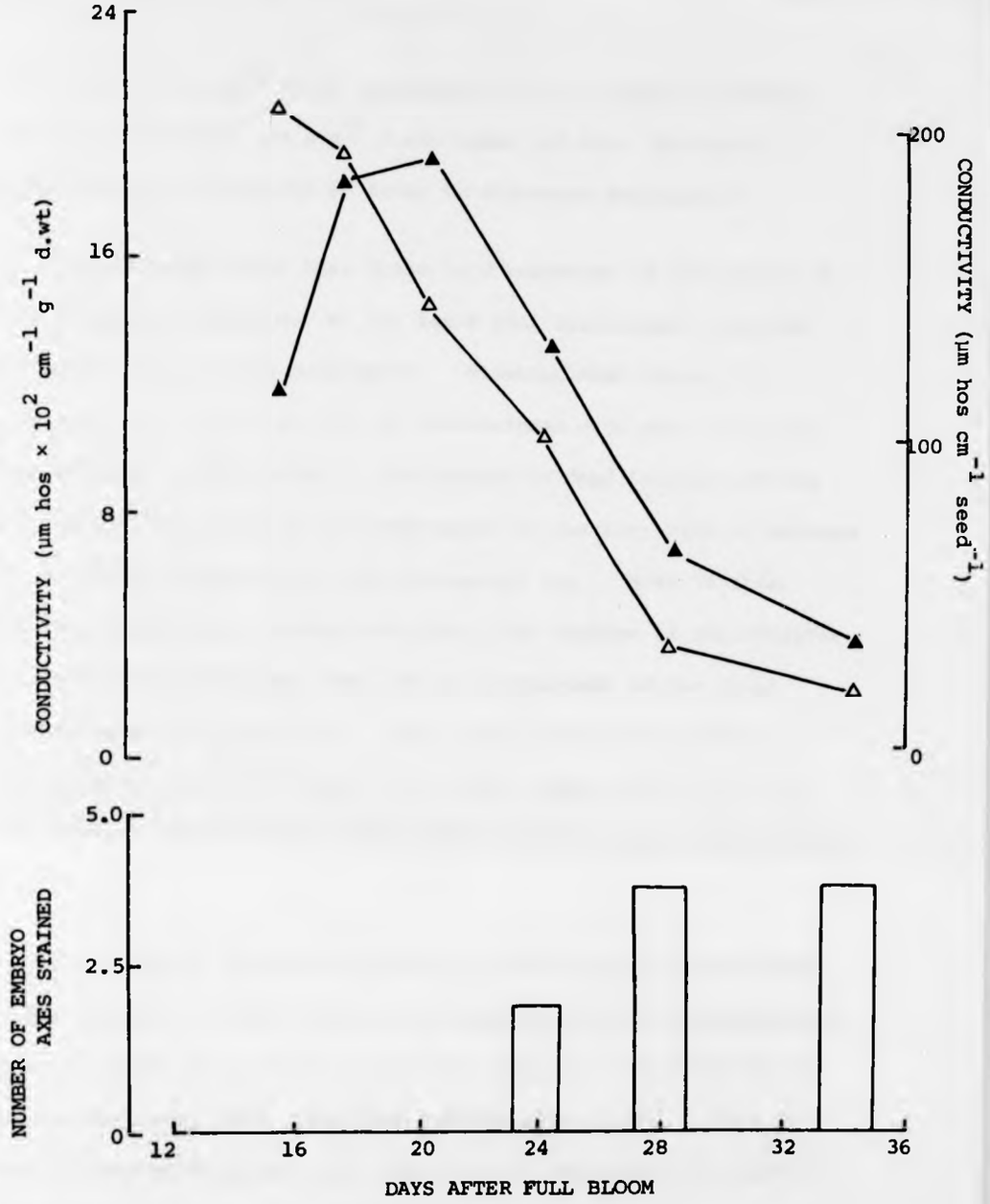

FIG.26 Changes during normal development in the 24h conductivity expressed g^{-1} d. wt. (Δ) and $seed^{-1}$ (▲) and in the viability of seeds subjected to vacuum desiccation.

fall seen on a $seed^{-1}$ basis coincides with the first significant fall in conductivity on a g^{-1} d.wt. basis and with the first appearance of the ability of seeds to withstand desiccation.

Having established that there is a reduction in the amount of electrolytes leaching out of dry seeds with age several possible explanations should be considered. Firstly, that there is a reduction in the availability of electrolytes with age; secondly that there are differences in the amounts of dead tissue involved and thirdly that there is an improvement in the retention of solutes by the cells of the embryo with increasing age. Work in this thesis suggests that during development the leakage of electrolytes, potassium ions and sugars over 24h is independent of the total amounts present in the seed. This idea of the independence of leakage from the amount present is further supported by work on mature pea seeds (Matthews and Rogerson, 1976; Powell and Matthews, 1977).

The level of leakage from dried mature seeds has been related to the amounts of dead tissue on the cotyledons with a predominance of dead tissue being a feature in seeds showing a high 24h conductivity (Matthews, 1971; Matthews and Rogerson, 1976). Work in this thesis would suggest that this was not completely the case in developing seeds. When dried seeds, harvested at different ages, were compared after tetrazolium tests the younger seeds had more dead tissue than the older seeds (Table 12). The conductivity of these younger seeds was also higher but cotyledons with the same amount of dead or living tissue did give very different levels of 24h leakage with older seeds leaking less. Thus, the differences in leaching seen cannot be explained in terms of either the avail-

ability of solutes to be leached or in terms of differences in the amount of dead tissue. This gives support to the suggestion that during development there is an improvement in the ability of the cells of the seeds to retain solutes.

One of the most obvious functions of cellular membranes is to physically compartmentalize the cell providing semi-permeable barriers between different compartments and, in the case of the plasmalemma, between the inside of the cell as a whole and the exterior. It is therefore reasonable to postulate that if there is an improvement in retention of solutes by cells that one of the possible ways of doing this is by improving the efficiency of the semi-permeable boundaries, in particular the plasmalemma, that controls solute movement.

Differences in the permeability of membranes have been attributed to the saturation of fatty acids, the nature of the hydrocarbon chains of the phospholipids, the interaction of sterols with phospholipids and the chemical nature of the polar headgroups of the phospholipids (Van Deenan et al., 1972). Therefore, if permeability can be influenced by changes in any one of these chemical constituents then the measurement of changes in any one constituent under set conditions could be expected to throw some light on improved retention of solutes by membranes. Using this line of reasoning, many people have looked at the fatty acid changes occurring under different circumstances in plant tissues and some of these changes have been associated with permeability changes. An example of this has been used to some effect in trying to understand the mechanism involved in chilling tolerance of plants and in this respect one of the factors that seem to be involved is that

of increased saturation of the fatty acids with hardening (Wilson and Crawford 1974a, b). In the same studies it was observed that with hardening there was also an increase in the total weight of phospholipids found. In deterioration studies on pea seeds Harman and Mattick (1976) found that as viability decreased (a time at which permeability was also decreasing - Powell and Matthews 1976) there was a reduction in the unsaturated fatty acids namely linolenic and linoleic acids. An increase in permeability has also been associated with a reduction is not only total phospholipids but in individual phospholipid species, in senescing cucumber cotyledons (Ferguson and Simon, 1973).

Work on membrane structures in developing seeds has been relatively little studies. There has been extensive study of fatty acid changes in developing oil seeds such as soybeans and castor beans (Appelquist 1975) but this has tended to be on the development of storage reserves rather than changes in the structure of membranes. However, in a general survey of lipids, during the development of soybeans Privett et al (1973) showed that generally there is an increase in both total phospholipids and in the major individual component phospholipids PG, PE, PC, PI and PA, this being more marked up to about 50 DAFB which on extrapolation of Ohmura and Howell's (1962) data on the respiration rate of soybeans would be about the peak in respiratory activity.

In developing pea seeds the major phospholipids found in this study were PC, PI and PG with PA and PE being found in comparatively minor amounts, a number of unknown phospholipids were also found. Quarles and Dawson (1969), in work on germinating pea seeds found that in the mature seed prior to germination the major phospholipids

were PC, PI and PE. During development, in succulent pea seeds, like developing soybeans there tends to be an increase in the total amount of phospholipids and in the PC and PI levels. However, unlike soybeans the levels of PG, PA and PE tend not to show any consistent pattern of change.

When changes in phospholipids were examined in relation to the development of the ability to withstand desiccation, as measured by an increase in solute retention and reduction in physiological activity, the most consistent changes in phospholipids were significant increases in the major phospholipids PC, PI and PG prior to the reduction in physiological activity. Patterns of change in PA are very difficult to resolve and this may be due to the dynamic nature of phospholipid turnover and the importance of PA as a precursor of many other phospholipids (Kates and Marshall, 1975). U_6 and U_7 are only found in the older seeds that are best able to withstand desiccation while phosphorus at the origin also tends to increase in these older seeds. It is difficult to come to any firm conclusions about the changes in phospholipids occurring prior to the development of the ability to withstand desiccation because of the marked influence of environment on the rate of development of pea seeds (McKee et al, 1955; Robertson et al, 1962) makes exact replication of any experiment when glasshouse grown material is used, difficult to achieve. This problem has been partially resolved by holding seeds in high humidity conditions which have been shown to prematurely bring about the development of the ability of seeds to withstand desiccation. When treated in this way the total levels of phospholipids, the levels of phosphorus at the origin, the level of PC and the levels of U_6 and U_7 increase. The exact

relevance of these changes to the ability of seeds to withstand desiccation is difficult to resolve but what these results do show is that changes in the chemical composition of membranes do take place during development and that these changes appear to be linked with the physiological events which enable the seeds to withstand drying to low moisture levels.

It has been established that phospholipid molecules have a very rapid turnover rate with the amount of any individual phospholipid species at any one time being the result of a balance between synthetic and degradation rates (Kates, 1970; McCullough and Simon, 1973; Moore, 1974, 1976; Donaldson, 1976). Any system that is so dynamic would be expected to have a constant requirement for an energy input and as such would be linked to the respiratory activity of the cell. On the basis of experiments using respiratory inhibitors, it has been suggested that with a reduction in respiration rate there is a reduction in phospholipids (Barker and Mapson, 1964; McCullough and Simon, 1973). However, it has also been postulated that an increase in membrane permeability during senescence is independent of the respiratory system (Eilam, 1965). In the present work the levels of phospholipids increase both when the respiration rate is increasing and when the respiration rate falls both naturally and under high humidity conditions. This would suggest that, at least over the time period studied, in developing pea seeds changes in phospholipids are not closely dependent on the respiratory system, if anything a reduction in respiration stimulates an increase in phospholipids.

This phospholipid turnover in developing seeds may only be tenuously linked with physiological events such as changes in

respiration rate but the important point about developing seeds is that they suddenly are able to withstand drying. Changes in the chemical composition of the membrane structures before this may help the seeds to withstand drying but the problem arises as to whether the actual process of drying has any effect on membrane structure.

It has been suggested that in the dry seed that membranes exist in a different physical phase due to the low hydration of the cells (Simon, 1974; Hendricks and Taylorson, 1976). Using this type of reasoning, if changes in hydration are too rapid then membrane architecture will be altered in such a way that changes are irreversible resulting in loss of viability. This could be one of the reasons why immature seeds are unable to withstand desiccation. It is also true that one of the features determining whether a membrane is capable of changing its physical state is the type of molecules present (Simon, 1974) therefore the actual chemical composition of the membranes in the dry seed compared to the succulent seed is important.

The results in this study suggest that in general, the pattern of phospholipid changes found in the dry seed are similar to those found in the succulent seed with two striking differences. Firstly, there is a marked reduction in the levels of phospholipids found in the dried compared to the succulent seed. One possible explanation of this could be that the extraction efficiency differs in succulent and dried material. Although this was not checked, the similarity between the levels of PE, phosphorus at the origin and U_6 in both succulent and dried seed and in the increase in U_7 in the dried seed would argue against this. Therefore, it would appear that

drying radically altered the phospholipid composition of the seed shown by a reduction in phospholipid phosphorus. The second striking difference between the succulent and the dried seed is the presence of four unknown phospholipids (U_1, U_2, U_3 and U_5) in the dried seed. Of these, three U_1, U_2 and U_3 decrease with an improvement in embryo condition. It is of interest that these three unknowns run between the origin and the most polar of the known phospholipids - PI. Thus, it can be suggested that these lipids are the result of a breakdown of other phospholipids on drying and in doing so they may cause damage to the membrane structure but that in seeds resistant to desiccation this damage will be much less severe. U_5 is again only found in dried seeds but its levels tend to increase with an improvement in embryo condition. It is possible to postulate that an increase in the level of U_5 which again might be a breakdown product may be beneficial rather than harmful to the maintenance of membrane integrity in the dried seed.

It can be postulated that the processes involved in drying alter the phospholipid structure in such a way that the molecules are disrupted as the seed dries, certainly membranes are incomplete structures in dried seeds (Buttrose and Swift, 1975). In the process of drying a certain amount of phosphorus simply disappears from the membrane system and it may well be that for a seed to withstand drying that there is a critical level of phospholipids that must be retained in membrane structure. This investigation, if nothing else, clearly shows that in trying to determine the properties of membranes in dried seeds the chemical characteristics of the membrane systems need to be much more extensively investigated.

Work in this thesis has tried to relate physiological changes

occurring during development with the ability of seeds to withstand desiccation. These observations can be used to try and describe the characteristics of seeds in the dry condition. This problem has been approached in at least two other ways; firstly, by attempting to look at the ultrastructure in the dry seed and secondly, by looking at changes very early on in imbibition to give some indication of the nature of the systems present in dried seeds.

When looking at the ultrastructure of the dried seed the immediate problem encountered is that of investigating a dry structure without hydrating it. Most of the techniques used to fix tissue prior to microscopical examination have involved some hydration. Swift and Buttrose (1973) have shown that even a ten second exposure to water will result in a structure being rehydrated. Light microscopic (Bain and Mercer, 1966) and electron microscopic studies (Klein and Pollock, 1968) that have used acqueous fixatives give a picture of membrane systems in the dry seed being extremely indistinct. Some attempts have been made to use non-acqueous fixatives such as fixing tissue in osmium tetroxide vapour (Perner, 1965) or by using usual fixatives in anhydrous glycerol (Hallan and Capicchiano, 1974) but the most successful method of looking at structures in the dried seed to date have been freeze fracture and scanning electron microscope studies. This has basically suggested that in the dry seed the membranes are incomplete structures with the bonds between the proetin molecules being most severely damaged by desiccation (Buttrose and Swift, 1976). This idea that the structure of the membranes in the dry seed is different from the structure in the succulent seed has further been suggested by changes in membrane structure being brought about by dehydrating and rehydrat-

ing membrane systems (Simon, in press). However a feature of membranes that has not been looked at previously has been the chemical constituents of membranes present in dry seeds. In this respect the most obvious feature of the dried when compared to the succulent state is that there is apparently a substantial reduction in the level of certain phospholipid components of membrane systems namely PC, PI, PG and PA, without a corresponding gain in any other phospholipids found. This raises the suggestion that for a seed to remain viable on drying there may be a minimal level of phospholipids necessary and it also raises the need for further investigation of phospholipid levels at different water contents.

During studies on changes during very early imbibition the most pronounced changes are those involved in respiratory metabolism. Yentur and Leopold (1976) have suggested that up to the first five hours of imbibition a cyanide-insensitive pathway is the main means of respiration after which the TCA cycle becomes increasingly important. This lack of activity in the TCA cycle very early in germination is further suggested by Lado (1965), Kollöffel (1969) and Kollöffel and Sluys (1970) who found in castor bean seeds and pea seeds that the activity of mitochondrial enzymes increases very slowly during the very early stages of imbibition. This being so, it would be reasonable to expect that the respiratory systems already present during very early imbibition would be present in the dry seed. However, when looking at changes during development up until the time when seeds first develop the ability to withstand desiccation (early phase 4 when using criterea of Bain and Mercer, 1966) there is no evidence for the presence of a cyanide-insensitive pathway. Thus if such a pathway is in operation in the dry seed

then it must only develop after the seed has lost a considerable amount of water.

Mitochondrial oxidation systems do not seem to be active during very early imbibition (Lado, 1965; Kolloffel and Sluys, 1970; Nawa and Asahi, 1971; Yentur and Leopold, 1976) and when looked at in developing seeds, not only does the oxidative activity of mitochondria decrease with development (Lado, 1965; Kolloffel, 1970a, b) but mitochondrial respiration appears to decline faster than those respiratory pathways taking place in the cytoplasm. However, by full maturity the respiration rate of dry seeds is barely measurable.

Thus by combining these three different ways of investigating the status of the dry seed a picture emerges of a seed high in storage reserves but low in moisture content, soluble carbohydrates, soluble nitrogen, DNA and RNA. The dry seed is also characterised by a low level of physiological activity especially in pathways associated with the mitochondria but less as far as pathways found in the cytoplasm are concerned. On an ultrastructural level, not only are membranes incomplete structures in dry seeds with a different physical architecture but there also appears to be a loss of membrane phospholipids on drying. Needless to say, this picture of the state of processes in a dry seed is by no means complete.

Germination of succulent seeds

It has been shown (Kidd and West, 1920; Matthews, 1973a; Eewens and Schwabe, 1975) that immature succulent seeds are capable of germination. Work in this thesis has shown that although very

young seeds at phase 2 in their developmental sequence (Bain and Mercer, 1966) are unable to germinate, succulent seeds from as early as the beginning of phase 3 in their development when the respiration rate is still rising can germinate. In contrast, seeds are only able to germinate after drying during phase 4 of their developmental sequence when the level of physiological activity has decreased. Thus succulent seeds are capable of germinating from an early stage in their development and yet they do not normally germinate while still in the pod.

Eewens and Schwabe (1975) have suggested that precocious germination of seeds is prevented by high concentrations of abscisic acid (ABA) and methyl-4-chloroindol-3yl acetate (MCIA) within the embryo. This conclusion is based on their finding decreasing amounts of these hormones associated with an increase in the coefficient of velocity of germination (CVG). Although referred to by Eewens and Schwabe (1975) as a measurement of germination capacity it is in fact a measure of the germination rate of viable seeds and differences in the CVG are not necessarily indicative of differences in germination capacity. It is possible that two samples of seeds could have similar CVGs but have very different germination percentages, e.g. Fig.21 seeds set to germinate plus and minus the testa at 25 DAFB have CUG's of 5.5 and 5.7 respectively but have corresponding percentage germinations of 10% and 68%. Thus from their results it can only be said that there is a decrease in certain hormone levels at the same time as there is an increase in the rate of germination of viable seeds.

It has also been suggested that early in development, germination of succulent seeds is prevented by the testa. Eewens and Schwabe (1975), again using the CVG as a measure of germination

capacity, concluded that removal of the testa did not influence germination capacity. However, as the CVG is not a measure of germination capacity this conclusion is not valid. Indeed, Kidd and West (1920) showed that removal of the testa resulted in an increase in percentage germination. In the present work, the germination capacity of immature seeds as measured by percentage germination was improved by an average of 40%, when the testa was removed. This was true until 50 DAFB by which time the respiration rate of the seeds was much reduced and removal of the testa did not affect the germination capacity of seeds which was itself high.

Kidd and West as long ago as 1920 suggested that germination may be prevented by the restriction of 0_2 supply to the embryo by the physical presence of the testa. Ohmura and Howell (1960) also found that the testa of soybeans restricted 0_2 movement much more than CO_2 movement while Wager (1974) found that 0_2 uptake in pea seeds was almost exclusively through the micropyle although 75% of the CO_2 given off by the seed is lost via the cuticle. In the present work the absence of the testa resulted in a significant increase in 0_2 uptake by embryos from 29 to 50 DAFB. This increase in respiration rate as a result of testa removal has also been shown in soybeans by Ohmura and Howell (1962).

Thus after 29 DAFB it is possible that germination of seeds is being prevented by restriction of the 0_2 supply to the seed. However, removal of the testa from seeds harvested at 25 and 27 DAFB did not result in an increase in 0_2 uptake. At these times the respiration of the testa (taken as the difference between the respiration rate of the entire seed and the embryo) was itself increasing

and it is likely that at 25 and 27 DAFB embryo respiration is restricted by competition with testa respiration. Before 25 DAFB succulent seeds were unable to germinate even without their testae.

Although the germination capacity of seeds at any age is increased by removal of the testa the overall capacity for growth, as measured by the CVG and radicle and plumule dry weights, shown by seedlings from young seeds is much less than that shown by seedlings from older seeds. In addition, seedling growth was the same from seeds of the same age whether the testa was present or not. During the period studied when removal of the testa improved germination the testa was thick and fleshy. It may be that in embryos held within the testa, although capable of some growth, the radicles were not able to grow sufficiently strongly to penetrate the testa. Thus precocious germination of succulent seeds may be prevented by a combination of a restriction of 0_2 supply and the strength of the testa but that these in themselves do not influence the potential vigour of the seedling which is low in immature seeds but high in mature seeds.

Eewens and Schwabe (1975) may not have shown that the germination capacity of seeds increases with age but they have shown that the germination rate of viable seeds does increase with age, i.e. that immature pea seeds germinate more slowly than mature seeds as shown by the CVG values. A similar situation has been found to occur in maize (Sprague, 1936). In the present work the germination rate of viable seeds as well as seedling vigour increases with increasing age of the seed. Many reasons can be put forward to explain this low growth potential of more immature seed and it may well be that the high levels of ABA and MCIA in immature seeds by Eewens and Schwabe (1975) may be involved.

During the course of this study it has been observed that immature seeds kept under high humidity conditions which result in a reduction in sugar content do not show a reduction in starch levels. It can be postulated that an embryo will only show a high growth potential if it is capable of fully utilizing its storage reserves. In pea seeds the main storage reserve is starch and in a developing seed starch is being synthesised whilst in the germinating seed starch degradation is taking place. In cereal seeds the degradation of starch has been firmly connected with the activity of α and β amylase (Evans et al., 1975) however, the situation is less clear in pea seeds. In the developing pea seed both α and β amylase activity is found very early in development when sugar levels are rising and starch synthesis is low (Turner and Turner, 1957) and as starch synthesis increases β-amylase activity declines markedly - it is not known what happens to α-amylase activity. During germination, amylase activity begins to increase two to three days after pea seeds are set to germinate Young and Varner (1959) and Swain and Dekker (1966) consider that α and β amylase activity is of increasing importance during the later stages of germination. This low level of amylase activity in the developing seed and the relatively long time required for an increase in amylase activity in the germinating seed would suggest that de novo synthesis of amylase enzymes are necessary before starch hydrolysis is possible. It has further been shown that the formation of α and β amylase in germinating pea seeds is inhibited by ABA (Yomo and Varner, 1973). Thus, if immature seeds have high levels of ABA as has been shown by Eewens and Schwabe (1975) then these seeds would be unable to synthesis amylase and as such would be unable to utilize the hydrolytic pathway for starch degradation and consequently would be unable to grow.

During the course of this study it has been observed that immature seeds kept under high humidity conditions which result in a reduction in sugar content do not show a reduction in starch levels. It can be postulated that an embryo will only show a high growth potential if it is capable of fully utilizing its storage reserves. In pea seeds the main storage reserve is starch and in a developing seed starch is being synthesised whilst in the germinating seed starch degradation is taking place. In cereal seeds the degradation of starch has been firmly connected with the activity of α and β amylase (Evans et al., 1975) however, the situation is less clear in pea seeds. In the developing pea seed both α and β amylase activity is found very early in development when sugar levels are rising and starch synthesis is low (Turner and Turner, 1957) and as starch synthesis increases β-amylase activity declines markedly - it is not known what happens to α-amylase activity. During germination, amylase activity begins to increase two to three days after pea seeds are set to germinate Young and Varner (1959) and Swain and Dekker (1966) consider that α and β amylase activity is of increasing importance during the later stages of germination. This low level of amylase activity in the developing seed and the relatively long time required for an increase in amylase activity in the germinating seed would suggest that de novo synthesis of amylase enzymes are necessary before starch hydrolysis is possible. It has further been shown that the formation of α and β amylase in germinating pea seeds is inhibited by ABA (Yomo and Varner, 1973). Thus, if immature seeds have high levels of ABA as has been shown by Eewens and Schwabe (1975) then these seeds would be unable to synthesis amylase and as such would be unable to utilize the hydrolytic pathway for starch degradation and consequently would be unable to grow.

The situation in pea seeds is however not quite so straight forward, in that in developing pea seeds an increase in starch synthesis is achieved by an increase in starch phosphorylase activity (Turner and Turner, 1957) while early in germinating seeds an increase in starch phosphorylase activity is associated with starch degradation (Swain and Dekker, 1966). This apparent inconsistency is explained by two phosphorylases being found in both developing and germinating pea seeds with the levels of phosphorylase II being higher and increasing with age in developing seeds synthesising starch while phosphorylase I increases rapidly in germinating seeds while phosphorylase II remains relatively constant (Matheson and Richardson, 1976).

The level of starch phosphorylase in germinating seeds increases before that of amylases (Swain and Dekker 1966; Matheson and Richardson, 1976) which suggests that early in germination starch degradation is brought about by phosphorylase I activity and not by amylase activity. Thus germinating seeds are capable of degrading starch before the hydrolytic breakdown of starch via amylase activity is fully functioned. As both phosphorylases are present in very immature seeds (Matheson and Richardson, 1976) even immature seeds have the potential for starch degradation and this could explain the observation of some, albeit reduced, growth found in the embryo axes of immature seeds in that some phosphorylase activity coupled with the low starch levels present in the immature seed would result in a limited amount of growth. However the problem arises as to how the switch from phosphorylase II being the predominant enzyme in a developmental situation to phosphorylase I being the predominant enzyme in a germination situation is brought about. In their work with barley Evans, Black and Chapman

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(1975) suggested that in barley grains starch degradation and consequently exploitation of the main energy store of the seed is only possible after some dehydration of the seed. In barley they found that dehydration is necessary for the development of complete sensitivity to gibberellin in the aleurone. Although in barley the main enzymes involved are amylases while in peas starch phosphorylases are indicated the necessity for some dehydration before starch degradation is possible would be expected to be of general evolutionary importance to most seeds.

In the present study the level of starch continued to increase during normal development as had previously been observed and there was no degradation of starch even seven days after seeds were set to germinate. Seeds that were subjected to either a three day drying treatment or a three day high humidity treatment again showed no degradation of starch. However seven days after these seeds were set to germinate starch degradation was observed. This degradation of starch cannot be explained by the drying treatment because starch degradation was also observed in the high humidity treated seed which had not been dried. The possibility that the difference between starch degradation being found in the seed that had been subjected to a three day treatment and those which had been set to germinate immediately, being simply the result of an extra three days in which metabolism could occur cannot be discounted. This would infer that after removal from the plant succulent seeds were always capable of degrading starch, the shift in the balance between the two phosphorylases being purely a function of time after the removal of the seed from the plant. If this is so, then the mechanism which would control the shift from one enzyme to

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another enabling starch degradation to occur still remains unresolved and attention should be given to the relationship between the parent plant and the seed.

This thesis has been about the physiological changes in developing seeds of one crop plant but some of the results have a wider significance. The evolution of a desiccated seed capable of withstanding unfavourable conditions and containing sufficient food reserves to facilitate seedling establishment has been a crucial factor in the success of the Angiosperms. An important factor in determining whether reproduction will be successful is the timing of flowering and subsequent seed development so that both take place under favourable conditions (Stebbins, 1971). In addition, the developing seed is protected from drastic changes in the environment such as drought, until the seed can withstand desiccation. It would be reasonable to suppose that if the seed is to be protected from premature dehydration, the sub-cellular changes in the seed essential for the tolerance of dehydration should be promoted not by the environment directly but indirectly by the influence of the parent plant. The work in this thesis enables just such a mechanism to be outlined.

In annual species such as peas, clear phases of senescence of the whole plant corresponding in time with the maturation and ripening of the fruit are found (Wareing and Seth, 1967; Carr and Pate, 1967). These changes are themselves under hormonal control (Wareing and Seth, 1967) but as the developing seed acts as a sink for many metabolites of carbohydrates, proteins, amides (Flinn and

Pate, 1970; Lovell and Lovell, 1970) then reduction in the supply of assimilates as a result of reduced availability fron senescing tissue would be one of the changes in the circumstances of a seed developing within the pod. A fall in the supply of sugars to the seed would bring about a fall in the level of physiological activity of the seed which is necessary for the seed tissue to withstand severe desiccation. In this way premature dehydration is avoided by the parent plant bringing about the necessary changes through its naturel senescence before the effect of the dry period reaches the seed.

During the formation of this resistant structure it would also be of adaptive value for the germination of the seed, before it has developed the ability to withstand adverse conditions, to be prevented. In the developing pea seed this appears to be associated with the physical strength of the testa, with the impermeability of the testa to 0_2 and with the inability of young embryos to degrade their starch reserves. In this way, not only is premature germination prevented, but the conservation of food reserves during development is also ensured. Thus, this investigation into physiological changes in developing pea seeds may have generated information of more general importance to our understanding of the evolutionary significance of the Angiosperm seed.

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APPENDIX I

Two unknown phospholipids extracted from the second harvest series

TABLE 1 : Levels of U_8 and U_9 found in succulent and dried pea seeds extracted immediately and after 3 days storage under 100% rh conditions

Treatment	DAFB	U_8		U_9	
		Initial	3 days 100% rh	Initial	3 days 100% rh
Succulent	20	0	0	0	0
	23	0	0	0	0
	25	0	144.4	127.8	0
	27	207.2	0	0	177.8
	30	161.1	0	0	0
Dried	20	192.6	144.4	161.1	144.4
	23	42.6	72.2	58.4	72.2
	25	125.9	42.6	142.6	42.6
	27	27.8	27.8	27.8	42.6
	30	0	0	0	0

U_8 and U_9 were found in phospholipid extracts from both succulent and dried embryos sampled freshly or after three days storage at 100% rh. These two unknowns were recognised because they ran directly beneath the solvent front and when present after development with iodine vapour they contained measurable amounts of phosphorus. The extremely variable findings of these unknowns in succulent embryos with the relatively high amounts found in the youngest dried embryos and the decreasing amounts found with age in the dried embryos would suggest that these were breakdown products. Their exact significance is not fully understood.