THE BIOCHEMISTRY AND PHYSIOLOGY OF
THE IMMATURE CEREAL PERICARP

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Declaration

This thesis was composed by myself and describes my own original work and has not been submitted for a degree at any other University.
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Abstract

The biochemistry and physiology of the immature cereal pericarp have been investigated. Immature detached cereal caryopses from two barleys and one wheat variety were shown to be capable of fixing externally-supplied radioactive CO$_2$. The cross cells/testa were found to contain the majority of the $^{14}$C-label. Some label was detected in the transparent layer and endosperm plus embryo. More $^{14}$C was recovered from caryopses of cv. Midas and Albino lemma, a mutant which has no chlorophyll in its cross cells, when incubated without their transparent layers. Thus it seems that the transparent layer is a barrier to the uptake of CO$_2$. The $^{14}$C-label found in the insoluble endosperm fraction was probably present mainly in starch and cell wall material. The results indicated that pericarp photosynthate is translocated to the endosperm where it may be incorporated into storage material.

The route taken by pericarp assimilates to the endosperm was investigated using microautoradiography. $^{14}$C-labelled assimilates were found in the outer tissues, the crease and the starchy endosperm of the caryopses. Label was also found in the walls of the phloem. Thus assimilates probably move round the grain in the outer tissues to the crease where they enter the endosperm via the nucellar projection.

Sucrose uptake by detached barley ears showed that little of the CO$_2$ respired by the endosperm was fixed by pericarp photosynthesis. However, pulse-chase experiments with $^{14}$CO$_2$ indicated that caryopses retained more $^{14}$C in the light than in the
dark thus suggesting that one function of the pericarp may be to reduce respiratory losses. The presence of PEP carboxylase activity in the pericarp supports this hypothesis.

In the light, starch synthesis in the endosperm of cv. Midas was stimulated but not in Albino lemma. This effect was attributed to oxygen production during pericarp photosynthesis. However, Albino lemma has a well-filled grain with a dry weight at maturity similar to that of cv. Midas and the lack of chlorophyll does not seem to affect grain filling.

The possible functions of the immature pericarp and its role in grain filling are discussed.
ABBREVIATIONS

ADP  Adenosine diphosphate
ADPG  Adenosine diphosphate glucose
ATP  Adenosine triphosphate
DHAP  Dihydroxyacetone phosphate
DPGA  Diphosphoglyceric acid
E4P  Erythrose 4-phosphate
FBP  Fructose bisphosphate
F6P  Fructose 6-phosphate
Hepes  (N-2-Hydroxyethylpiperazine-N'-2-ethanesulphonic acid)
G1P  Glucose 1-phosphate
G3P  Glyceraldehyde 3-phosphate
G6P  Glucose 6-phosphate
MOPS  2-(N-morpholino)ethane sulphonic acid
NADH/NADPH  Reduced nicotinamide adenine dinucleotide
OAA  Oxaloacetic acid
Pi  Orthophosphate
PEP  Phosphoenol pyruvate
PGA  Phosphoglyceric acid
PSCR  Photosynthetic carbon reduction cycle
RPP  Reductive pentose phosphate
RuBISCO  Ribulose bisphosphate carboxylase/oxygenase
RuBP  Ribulose bisphosphate
R5P  Ribose 5-phosphate
SBP  Sedoheptulose bisphosphate
Sucrose-P  Sucrose phosphate
S7P  Sedoheptulose 7-phosphate
TCA  Tricarboxylic acid
TP  Triose phosphate
Tricine  (N-Tris(Hydroxymethyl)methyl glycine)
UDP  Uridine diphosphate
UDPG  Uridine diphosphate glucose
UTP  Uridine triphosphate
Xu5P  Xylulose 5-phosphate
1.1 The Importance of Cereals to Agriculture

The Gramineae, or grasses, form one of the largest plant families, members of which are found in every region of the world. Included in this classification are the cereals which are cultivated intensively and are economically very important. Both wheat and barley are thought to have been domesticated by man in Syria, Turkey and Iran about 7000 B.C. (Briggs, 1978). Maize, millet, rice and wheat form the basis of the diet of millions of people and barley and maize are also used extensively for animal production (Langer and Hill, 1982).

Table 1 Major Food Crops (FAO Production Yearbook, 1980)

<table>
<thead>
<tr>
<th>Crop</th>
<th>World Production</th>
<th>Contribution to Total Production (%)</th>
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<tbody>
<tr>
<td>Wheat</td>
<td>417</td>
<td>15.7</td>
</tr>
<tr>
<td>Rice</td>
<td>345</td>
<td>13.0</td>
</tr>
<tr>
<td>Maize</td>
<td>334</td>
<td>12.5</td>
</tr>
<tr>
<td>Barley</td>
<td>190</td>
<td>7.1</td>
</tr>
<tr>
<td>Oats</td>
<td>49</td>
<td>1.8</td>
</tr>
<tr>
<td>Rye</td>
<td>28</td>
<td>1.0</td>
</tr>
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</table>

Cereal grains are one of the principal raw materials used in alcoholic fermentation and in the United Kingdom much of the barley produced is sold to the malting industry.
1000's tonnes

<table>
<thead>
<tr>
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<th>1980</th>
<th>1981</th>
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<tr>
<td>Malting and distilling</td>
<td>1880</td>
<td>2005</td>
</tr>
<tr>
<td>Animal feed</td>
<td>3575</td>
<td>2395</td>
</tr>
<tr>
<td>Seed</td>
<td>285</td>
<td>310</td>
</tr>
<tr>
<td>Pearl and pot barley</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Waste</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>Exports</td>
<td>2170</td>
<td>3800</td>
</tr>
<tr>
<td>Total</td>
<td>7970</td>
<td>8600</td>
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</tbody>
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1.2 The Structure of a Cereal Ear

The inflorescence or ear of wheat is composed of a central spike or rachis with spikelets positioned at nodes on opposite sides of the rachis (Fig. 1.1.a). Each spikelet consists of a group of two to nine florets of which the central florets are frequently sterile. Non-flowering glumes subtend the spikelet (Fig. 1.1b). The florets are joined to one another by a short rachilla which has vascular connections with the rachis. Assimilates produced in the leaves and stem of the plant enter the grains via the vascular tissue of the rachis and rachilla.

The grain of wheat is described as naked, since at maturity the husk does not adhere to the caryopsis. In contrast, the mature grain of most barleys consists of a caryopsis tightly enclosed by the husk. However, some naked barleys are grown in Asia for human consumption (Briggs, 1978). The
Fig. 1.1  a) Ear of wheat  b) wheat spikelet  c) ear of 2-row barley  
d) barley spikelet  e) ear of 6-row barley  
f) barley spikelet.
barley ear has a structure similar to that of wheat, the main difference being in the arrangement of grains on the rachis. Three spikelets are possible at each node and if all three are fertile then the variety is a six-row barley (Fig. 1.1e). If only the median floret is fertile the variety is described as two-rowed (Fig. 1.1c). Each spikelet is subtended by two non-flowering glumes (Fig. 1.1d and f). The rachilla extends above the base of the floret and lies in the lower part of the crease or groove of the grain. The palea is partly enclosed by the lemma and together these tissues comprise the husk. Awns, which are extensions of the lemma, may also be present.

1.3 Developmental Morphology of the Grain

The cereal grain undergoes major changes in the period from anthesis to maturity (harvest ripeness). In the United Kingdom, grains reach maturity about 60 to 70 days after anthesis (Laberge et al., 1971). During this time the grain enlarges considerably and storage material is laid down in the endosperm for use by the embryo during germination. During early grain development the pericarp, which is derived from the ovary wall, constitutes the principal component of a barley caryopsis (Jennings and Morton, 1963). A caryopsis is a grain minus the husk. Not until about 16 days after anthesis does the endosperm account for more than 50 percent of the total dry matter. At maturity the endosperm constitutes about 80 percent of the total dry matter (MacMasters et al., 1971) and consists mainly of starch, storage proteins and lipids.
1.3.1 The Glumes

The lemma and palea which comprise the husk, and the sterile glumes which subtend the grain, together are termed the glumes. At maturity the lemma and palea are tightly fused together in barley. In contrast, the glumes of wheat loosely cover the grain. At an early stage of development the lemma of wheat partly encloses the palea (Bremner and Rawson, 1972). The fertile glumes have a structure similar to that of the leaf sheath and contain chlorophyll during early grain development.

A few stomata have been identified on the exterior surface of the palea of barley (Miskin and Rasmusson, 1970). No stomata were found on the lemma but there were two rows on the awn. Percival (1921) noted the presence of stomata on both surfaces of the lemma of wheat and rows of stomata on the outer face of the awn. A few stomata were found over the palea.

Both barley and wheat may be awned or awnless. The awn is an extension of the top of the lemma and is also chlorophyllous.

1.3.2 The Pericarp

Until about seven days after anthesis the growth of the cereal caryopsis is largely accounted for by the enlargement of the pericarp and testa (Harlan, 1920). The pericarp is of maternal origin and is derived from the ovary wall.

At anthesis the outer layer of the barley pericarp is composed
of a single layer of epidermal cells and thin walled parenchymatous cells which are vacuolate and contain small starch granules (Esau, 1953). Initially, the cells are white and opaque but as the grain develops the cells become transparent as a result of the disappearance of the starch granules. Hydrolysis of the starch in barley starts early in development (May and Buttrose, 1959) and the starch has disappeared by about 14 days after anthesis in Canadian grown barley (MacGregor et al., 1972).

As the caryopsis matures the epidermis becomes compressed and the parenchymatous cells start to degenerate, leaving an air space between the broken cell walls and the inner layer or cross cells of the pericarp. This process starts at 2 to 3 days after anthesis in barley (M.P. Cochrane, personal communication). At 25 days after anthesis the transparent layer has 4 to 5 cell layers except at the crease where there are usually 2 cell layers (Cochrane and Duffus, 1979). At maturity the transparent layer of wheat consists mainly of compressed cell walls (Percival, 1921).

Stomata have been found in the epidermis of both wheat and barley (Bradbury et al., 1956a; Cochrane and Duffus, 1979; Gifford and Bremner, 1981a). Up to 10 stomata are situated on the ventral side at the brush end of the barley caryopsis and more than 30 can be found in wheat. Between 15 and 30 days after anthesis, the tip of the caryopsis of both barley and wheat is only loosely covered by the fertile glumes, thus exposing the stomata to the atmosphere.
The inner epidermis of the pericarp, and the layer of cells next to it differentiate, to form tube cells and chlorophyll containing cells (Percival 1921; Krauss, 1933). Tube cells and cross cells have been reported to be unique to grass caryopses (Rost and Lersten, 1973). As the caryopsis expands, the inner epidermis disintegrates and at 15 to 30 days after anthesis the remaining cells are widely spaced tube cells at the dorsal side of the caryopsis (Cochrane and Duffus, 1979). These cells are elongated parallel to the long axis of the grain. Wheat tube cells contain proplastids at anthesis, some of which become chloroplasts or amyloplasts (Morrison, 1976). By 17 days after anthesis these cells have lost their starch and cytoplasm.

The cells outside the tube cells elongate in a plane at right angles to the long axis of the grain and are known as cross cells. They extend round the seed to the crease where the number of cells increases considerably, resulting in thick green strands at either side of the vascular tissue. The cross cells form a continuous band which is 2 to 3 cells thick in barley, and is one cell thick in wheat, oats, rice and rye. These cells contain chloroplasts and the chlorophyll content of barley cross cells reaches a maximum 27 to 30 days after anthesis and then declines to almost zero at 40 days after anthesis (Duffus and Rosie, 1973). The chloroplasts of wheat and barley contain starch until midway through the grain filling period (Morrison, 1976; Williams, 1976; Cochrane and Duffus, 1979).

Plasmodesmata have been found between cross cells of the same
layer in barley (Cochrane and Duffus, 1979), wheat (Morrison, 1976) and rice (Hoshikawa, 1969). No plasmodesmata have been observed between the cross cells and the testa (Cochrane and Duffus, 1979).

1.3.3 The Testa and Nucellus

The integuments and nucellus undergo more extensive changes during grain development than the ovary wall. As the embryo sac enlarges after fertilisation, the outer integument is reabsorbed. By 2 to 3 days after anthesis the inner integument abuts the tube cells of the pericarp of wheat (Morrison, 1976). This integument, which gives rise to the testa or seed coat, is composed of 2 layers of cells crossing each other at an angle less than 45 degrees (Bradbury et al., 1956a). The testa is continuous round the caryopsis to the crease region where it merges with a pigment strand or chalaza.

A thick cuticle is present on the outer layer of cells of the testa of barley and wheat (Brown, 1907; Collins, 1918; Pugh et al., 1932; Krauss, 1933; Morrison, 1975; Cochrane and Duffus, 1979). The cuticle is continuous round the grain except at the crease and at the micropyle. At maturity, the cuticle penetrates between the cross cells and tube cells, thus attaching the seed to the pericarp (Netolitzy, 1926).

During early development, the nucellus of the ovule is used as a nutrient by the expanding endosperm and embryo. Only the nucellar epidermis and a band of cells running parallel to the chalaza persist during the grain filling period. This
band of cells, termed the nucellar projection by Krauss (1933), develops thickened cell walls as the grain matures and cells with the characteristics of transfer cells are found in this tissue in barley (Cochrane and Duffus, 1980). By 33 days after anthesis the nucellar epidermis is compressed to a hyaline layer round the endosperm. A thin cuticle of nucellar origin adheres to the testa and is continuous round the grain except at the crease (Morrison, 1976; Cochrane and Duffus, 1979).

1.3.4 The Crease

The crease tissue extends the length of the grain in the furrow at the ventral side. A vascular bundle consisting of phloem elements lying in an arc surrounded by phloem parenchyma runs the length of the furrow. Small groups of xylem vessels are found in the phloem parenchyma. At the base of the grain the phloem is continuous with that of the rachilla but the xylem is discontinuous (Zee and O'Brien, 1971a). Two vascular bundles are also present at the lateral sides of the caryopsis.

Inside the xylem is the chalaza, the cells of which contain increasing amounts of phenolic substances from 15 days after anthesis (Cochrane, 1983). From 35 days after anthesis the walls of these cells become suberized and lignified. By 55 days after anthesis the cell contents are disorganised and at 60 days after anthesis the cells are crushed.

At 7 days after anthesis the nucellar projection of barley which runs parallel to the chalaza has thin-walled cells except those bordering the endosperm cavity which have thick walls
By 10 to 15 days after anthesis, transfer cells are found in the nucellar projection, close to the endosperm cavity. Between these transfer cells and the endosperm cavity are cells with thickened cell walls which degenerate and the cells outside them elongate and in turn acquire the characteristics of transfer cells. At the end of the grain filling period only the cells in the core of the nucellar projection retain the characteristics of transfer cells. The massive wall thickening of cells bordering the endosperm cavity may be callose (Cochrane and Duffus, 1980).

1.3.5 The Endosperm

Immediately before anthesis the embryo sac contains one egg cell, 2 synergids and 2 polar nuclei near the micropyle (Esau, 1953). An outer and inner integument, both 2 cells thick, surround nucellus tissue which in turn surrounds the embryo sac. At fertilisation, pollen is released from the anthers and enters the sac via a pollen tube which grows through the micropyle. One male gamete fuses with the 2 polar nuclei giving rise to the triploid endosperm. The primary endosperm nucleus undergoes several divisions before the second fertilisation event, the fusion of the egg cell with the other male gamete, resulting in the diploid zygote.

Following the stage of free nuclear division, cell walls ingrowths furrow in from the edge of the embryo sac 1 to 2 days after anthesis (Morrison and O'Brien, 1976). These walls
branch and meet. Cytokine sis in this layer then produces all the subsequent cells of the endosperm. However, Mares et al. (1977) did not observe this branching from the sac wall.

Cellularisation of the wheat, barley, rye and maize endosperms is complete 4 to 5 days after anthesis. In the wheat endosperm, cell division ceases between 14 and 20 days after anthesis (Evers, 1970; Wardlaw, 1970; Briarty et al., 1979) whereas in barley, cell number increases up to 30 days after anthesis (Cochrane and Duffus, 1981). The peripheral cells become the aleurone layer. Differentiation of aleurone cells begins before cell division stops and these cells are distinguished from the starchy endosperm cells by the presence of lipid material and aleurone bodies. In barley the aleurone is 3 to 6 cells thick whereas wheat has a single layer.

Plasmodesmata exist between aleurone cells of the same layer and between aleurone cells and the starchy endosperm cells. The starchy endosperm also has plasmodesmata between cells. Starch accumulates in these cells when cellularisation is complete and is contained as granules in amyloplasts. Protein accumulates in membrane bound vesicles in the endosperm.

1.3.6 The Embryo

After at least 4 divisions have taken place in the endosperm, the first divisions of the zygote occur and give rise to the proembryo which has a suspensor cell embedded in the nucellus. By 7 days after anthesis, modified endosperm cells surround the wheat embryo (Smart and O'Brien, 1983). Between 15 to 30
days after anthesis the embryo grows rapidly and differentiation of the coleoptile, shoot and root primordia and coleorhiza are initiated. The mature embryo lies at the dorsal side at the base of the grain next to aleurone or crushed nucellar epidermal tissue (Bradbury et al., 1956b).

No plasmodesmata have been observed between the embryo and endosperm of barley or wheat (Norstog, 1972; Smart and O'Brien, 1983).

1.4 The Importance of Ear Photosynthesis to Grain Filling

Carbohydrate in the grain of cereals is thought to be largely derived from photosynthesis during the grain filling period (Archbold, 1942, Thorne, 1966) although carbohydrate stored in the stem prior to anthesis may contribute to final grain weight especially under stress conditions (Biscoe et al., 1976; Austin et al., 1977; Badinger et al., 1977; Stoy, 1979, 1980.) The major source of assimilate for grain filling is probably current photosynthesis by the flag leaf, the stem immediately below the ear, and the ear itself (Quinlan and Sagar, 1962; Carr and Wardlaw, 1965; Thorne, 1965; Lupton, 1966; Puckeridge, 1968).

Estimates of the contribution by ear photosynthesis to grain yield depend on the cultivar, the growth conditions, and the method used. Thorne (1966) reviewed the techniques used to measure the photosynthetic activity of cereal ears. The four most commonly used techniques are: shading of photosynthetic organs; radioactive carbon dioxide fixation; excision of
Shading of the ear either at ear emergence or after ear emergence can give an estimate of the contribution by the ear. Values of 15 - 39 percent (Thorne, 1963) and 26 percent (Watson et al., 1958) for the contribution to yield by barley ear photosynthesis have been measured but are probably underestimates as removal of a photosynthetic source is compensated for by increased contributions from other sources (Birecka and Dakic-Wlodkowska, 1964; Puckeridge, 1968). This technique does not take into account any respiration by the shaded organ.

Radioactive carbon dioxide ($^{14}$CO$_2$) fixation by different plant parts has been used to demonstrate which plant organs can supply assimilates to the grain (Buttrose and May, 1959; Quinlan and Sagar, 1962). Measurements obtained with this technique suggested that between 33 percent and 50 percent of the assimilates accumulated in barley grains were supplied by CO$_2$ fixation by the ear (Birecka et al., 1964). Similarly at mid grain filling 50 to 60 percent of the total carbon assimilation in intact plants could be accounted for by CO$_2$ fixation in the inflorescences of Lolium perenne L. and Poa annua (Ong et al., 1978). Removal of barley grains and shading of the ear in conjunction with $^{14}$CO$_2$ fixation showed that halfway through the grain filling period 43 percent of the yield was provided by ear photosynthesis (Buttrose and May, 1959). Using a similar technique Kriedemann (1966) estimated the contribution by the wheat ear was 29 percent. However, removal of grains can alter the balance of both the source and the sink as the contribution by other organs may increase to compensate for the absence of
Using gas exchange analysis the values for ear contribution to yield were estimated to be 17 percent for barley (Thorne, 1965), 33 percent for awned wheat and 20 percent for awnless wheat (Evans and Rawson, 1970) and 34 percent for barley (Porter et al., 1950). In general it seems that photosynthesis by the barley ear makes a greater contribution to yield than does the wheat ear.

1.4.1 Contribution to grain filling by grain photosynthesis

In 1965 Thorne suggested that the green tissues of the grain could supply much of the carbohydrate needed for grain filling. Individual chlorophyll containing components of the grain have photosynthetic ability (Carr and Wardlaw, 1965; Evans and Rawson, 1970; Duffus and Rosie, 1973; Wirth et al., 1977; Nutbeam, 1978) and the grains of the grasses Lolium perenne L. and Poa annua also can fix CO₂ (Ong et al., 1978). The assimilates produced by the tissues of the grain could be used in the synthesis of storage material in the grain.

1.4.2 Contribution by the Glumes

The positive influence of awns on the yield of cereals has been described by Vervelde (1953) and it has been shown that ear photosynthesis is greater in awned cereal varieties (Buttrose and May, 1959; Carr and Wardlaw, 1965; Evans and Rawson, 1970; Olugbemi et al., 1976). Eighty percent of the total ear CO₂ fixation was attributed to the awns of the large awned Triticum durum...
and Triticum turgidum species (Olugbe mi et al., 1976). However, light interception by the awns may decrease photosynthesis in the remainder of the ear. In turn, lower rates of photosynthesis may be compensated for by increased assimilate supply from the leaves and stem.

Although the contribution by the awns to yield may not be large, 10 percent for spring barley, 3 to 5 percent for wheat and less than 2 percent for rye (Lamb, 1937; Vervelde, 1953), it seems that at high temperatures or in drought conditions the presence of awns may be advantageous (Evans et al., 1972). Awned wheat varieties are also well adapted to warm dry climates (Vervelde, 1953). The awns have a high transpiration rate which could increase the flow of assimilates to the grain. However, in the United Kingdom, crops are in general not subject to drought and so it may not be advantageous to grow awned varieties.

In some cases, the presence of awns has been shown to detrimental. Yield was lower with awned wheat varieties (Mackenzie, 1972). It seems likely therefore that the effect of awns on yield is dependent on the environment in which the varieties are grown. Comparisons between awned and awnless varieties are not useful unless the genetic backgrounds of the varieties are the same. Any variations in yield could be attributed to a range of physiological differences.

The sterile glumes which subtend the grain of wheat and the fertile glumes, the lemma and palea, are capable of photosynthesis. Of all the glumes, the lemmas of an awned wheat variety were photosynthetically most active and accounted for
15 percent of whole plant photosynthesis over the greater part of the grain filling period (Bremner and Rawson, 1972). The sterile glumes and palea accounted for 7 to 11 percent. The glumes of _Lolium perenne_ L. fixed two-thirds of the total carbon assimilated at mid grain filling (Ong _et al._, 1978). Thus the glumes make a considerable contribution to ear photosynthesis and may therefore be major contributors to grain yield.

1.4.3 Contribution by the Pericarp

The cereal caryopsis has been shown to be capable of light-dependent carbon dioxide fixation and oxygen evolution (Wirth _et al._, 1977; Nutbeam and Duffus, 1978). Photosynthesis by the caryopsis of the awned wheat cv. Sonora was 31 percent of gross ear photosynthesis and was 43 percent for the awnless wheat cv. Gabo (Evans and Rawson, 1970). However, dark respiration by the caryopsis was about equivalent to gross photosynthesis throughout the rapid grain filling period. One wheat cultivar, cv. Pitic showed net photosynthesis until nine days after anthesis. Nutbeam (1978) suggested on the basis of oxygen exchange measurement that the barley pericarp is capable of fixing up to 30 percent of the grain carbohydrate. Photosynthesis by the caryopsis may therefore act as a source of metabolites and energy for the grain during rapid grain filling. This may compensate for the losses due to the high rate of grain respiration (Carr and Wardlaw, 1965).

The chlorophyll containing cross cells of the immature pericarp are presumably the site of caryopsis photosynthesis. However,
much speculation surrounds the possible role of pericarp photosynthesis in grain filling. It is not clear, for example, whether the CO₂ fixed is derived from the atmosphere or from endosperm respiratory processes or both. The fate of any evolved oxygen is unknown.

1.5 Biochemistry of Grain Development

1.5.1 Carbon Dioxide Fixation

Plants obtain carbon for growth and storage through photosynthesis by fixation of atmospheric carbon dioxide. The photosynthetic carbon reduction cycle (PSCR Cycle) is the biochemical pathway whereby CO₂ is reduced to sugar phosphates in all higher plants (Bassham et al. 1954; Bassham and Calvin, 1957; Norris et al., 1955). The enzymes catalysing the reactions of the pathway are located in the stroma of the chloroplasts. The product of the PSCR Cycle is triose phosphate which can be converted to starch in the chloroplast via a series of enzymic conversions resulting in the transfer of a molecule of ADP glucose to a primer thus forming unbranched α(1→4)-linked polymers such as amylose. The triose phosphate can also re-enter the PSCR Cycle thus regenerating ribulose bisphosphate, the CO₂ acceptor, or it can be exported to the cytosol where sucrose is synthesised (Fig.1.2).

In photosynthesising tissue, CO₂ may also be evolved as a result of photorespiratory and respiratory processes. In C₃ plants photosynthesis is accompanied by a loss of CO₂, termed photorespiration. C₄ plants may or may not exhibit photorespiration. It is considered that the CO₂ evolved during photorespiration
Fig. 1.2 Photosynthetic carbon reduction cycle
Fig. 1.3 $C_4$ Photosynthesis in a Leaf

1. PEP carboxylase
2. Malate dehydrogenase
3. NADP Malic enzyme
   - NAD malic enzyme
   - PEP carboxykinase
4. Pyruvate orthophosphate dikinase
is produced during the further metabolism of glycollate, which in turn is a product of the oxygenase activity of ribulose biophosphate carboxylase/oxygenase (RUBISCO). Oxygen competes at the active site and instead of two triose phosphates, only one is produced with a molecule of phosphoglycollate.

In some plants there is an additional pathway of CO₂ fixation, the C₄ or Hatch-Slack pathway, (Fig.1.3) whereby CO₂ is first incorporated into four carbon acids and is later released to be refixed via the reductive pentose phosphate pathway (Kortchak et al., 1965; Hatch and Slack, 1966, 1970). The productivity of C₃ plants has been stated to be lower than that of C₄ plants (Zelitch, 1975). However, Gifford (1974) noted that there was little difference in growth rates if plants were grown in their preferred environments.

The presence of Kranz leaf anatomy has been used to distinguish C₄ and C₃ plants (Downton, 1971) but is not considered to be definitive, as variations in anatomy exist in plants described as C₄ as well as C₃ – C₄ intermediate types. However, the presence of two green cell types is considered to be necessary for C₄ photosynthesis with spatial separation of the C₄ carboxylation and decarboxylation between two cell types. The cereal pericarp has only one green cell type. It has also been noted that in species with characteristics intermediate between C₃ and C₄ plants, the carbon isotope composition is similar to that of C₃ plants. Willmer and Roksandic (1980) found this was the case for barley and oat tissues. They suggested that under atmospheric conditions direct fixation
of external CO$_2$ through PEP carboxylase was relatively small compared to fixation through RUBISCO.

In the barley pericarp the ratio of PEP carboxylase to RUBISCO activity is high and similar to that found in maize leaves, a C$_4$ plant (Nutbeam, 1978). However, the ratio in wheat pericarps was lower and the products of the reductive pentose phosphate cycle predominated (Wirth et al., 1977).

Considerable variation in biochemistry exists within the classification of C$_4$ plants. Three groups have been distinguished based on the enzymes of the decarboxylation mechanisms in the bundle sheath cells. These are PEP carboxykinase, NADP- and NAD-malic enzymes (Edwards et al., 1971; Hatch, 1971; Hatch and Kagawa, 1974a, 1974b). In all three subgroups there is a similar localisation of enzymes in the mesophyll for the metabolism of pyruvate and CO$_2$ to malate. The enzymes are situated in the chloroplasts with the exception of PEP carboxylase. The location of PEP carboxylase was uncertain but using maize protoplasts it has been shown to be present in the cytoplasm (Gutiérrez et al., 1975).

Wheat and barley are regarded as C$_3$ plants since their leaves fix atmospheric CO$_2$ via the PSCCR cycle. However, immature wheat and barley pericarps contain a number of enzymes characteristically associated with the pathways of C$_3$ and C$_4$ photosynthesis (Duffus and Rosie, 1973; Wirth et al., 1977; Nutbeam, 1978). These include PEP carboxylase, NAD and NADP malate dehydrogenase, NADP malic enzyme, pyrophosphatase, transaminases and pyruvate orthophosphate dikinase. Meyer et al. (1982)
also found pyruvate orthophosphate dikinase in the green grains of eight species but suggested that the enzyme was located in the aleurone of the grains.

The first-formed product of photosynthesis in immature barley cross cells is the C₄ dicarboxylic acid malate. As with other photosynthetic tissues however, sucrose is the major end-product of CO₂ fixation (Nutbeam and Duffus, 1976). The pericarp thus has some unusual properties which may be related to its situation, surrounding the developing endosperm and embryo.

1.5.2 Sucrose Metabolism in Leaves

Sucrose is probably the major transport material in all higher plants (Porter, 1962) including cereals, although the phloem contents of these plants have not been analysed. The availability of sucrose for transport from the source (green cells) to the sink (grain) in cereals is governed by many factors including the rate of photosynthesis; the partitioning of fixed carbon between starch and sucrose; the rate of sucrose synthesis; the compartmentation of sucrose between transport and non-transport pools; and the translocation of sucrose out of source cells into the phloem.

The results of many investigations into the biochemistry of sucrose synthesis led to the conclusion that UDP glucose and fructose-6-phosphate are the precursors of sucrose phosphate which in turn is the immediate precursor of sucrose (Fig. 14). The final step is catalysed by sucrose phosphate
Fig. 1.4 Sucrose and starch synthesis in the leaf cell

1. Fructose bisphosphate aldolase
2. Fructose bisphosphatase
3. Glucosephosphate isomerase
4. Phosphoglucomutase
5. UDP glucose pyrophosphorylase
6. Sucrose phosphate synthase
7. Sucrose phosphate phosphatase
8. ADP or UDP glucose pyrophosphorylase
9. Starch synthase
synthase which has been found in both C₃ and C₄ leaves (Whittingham et al., 1979). The site of sucrose synthesis has been shown to be the cytoplasm and not the chloroplast (Bird et al., 1974). UDP glucose pyrophosphorylase was also found in the cytoplasm as was sucrose phosphatase. In the C₄ plant maize, enzymes of sucrose synthesis have been identified in both the mesophyll and bundle sheath cells (Downton and Hawker, 1973).

1.5.3 Starch Metabolism in Leaves

Within the leaf chloroplast, the triose phosphate product of the PSCR cycle can be used for starch synthesis (Ghosh and Preiss, 1965). The conversion of glyceraldehyde phosphate and dihydroxyacetone phosphate to hexose phosphate followed by the synthesis of nucleotide sugars takes place in the stoma of the chloroplast (Fig. 1.4). In leaves the ADP glucose pathway is predominant (Preiss and Levi, 1979). Once formed, the nucleoside sugar can transfer a glucose molecule to lengthen the amylose chain of a starch molecule. The synthesis of the α-(1,6)-linkages found in amyllopectin is catalysed by branching enzymes.

Starch synthesis and degradation is thought to be more closely regulated in photosynthetic tissues than in storage tissues where the synthesis of starch predominates. Starch synthesised during the day in leaves can be totally degraded during the night.
The most important change that occurs during the development of cereal grains is the synthesis of starch and its deposition in the endosperm. Just prior to fertilisation the ovule is small and has no endosperm or embryo. After fertilisation as the grain develops, tissue differentiation takes place and starch is synthesised in the starchy endosperm cells. Sucrose is thought to be the major carbon source for starch synthesis in reserve tissues such as the cereal endosperm (Duffus, 1979; Jenner, 1982). The amount of starch synthesis in the endosperm may be controlled by photosynthate production in the source tissues, the transport process from the source to the grain, and the rate of sucrose entry into the endosperm cells. Within the cells the enzymes of sucrose and starch metabolism could control the amount of starch synthesised.

Starch, which consists of amylose and amylopectin, is stored in the endosperm in amyloplasts. These plastids contain granules surrounded by stroma and are enclosed by a double membrane. In wheat, amyloplasts are present when endosperm cellularisation takes place (Morrison and O'Brien, 1976). The amyloplast population can be subdivided into large or A-type and small or B-type amyloplasts (Evers, 1971). A-types are found throughout development whereas B-type granules have been identified from 14 to 24 days after anthesis (Williams and Duffus, 1977).

Sucrose degradation and subsequent starch synthesis in the endosperm cells have been described by Duffus (1979) and
Duffus and Cochrane (1982). Their proposed scheme for the conversion of sucrose to starch is shown in Fig. 1.5. This scheme takes no account of amyloplast membrane permeability. In the cytoplasm, sucrose can either be converted to glucose and fructose by invertase (E.C. 3.2.1.26) or to the sugar nucleotides ADP glucose or UDP glucose and fructose by sucrose synthase (E.C.2.4.1.13). In the barley endosperm, invertase has a minor role with most of the incoming sucrose hydrolysed by UDP dependent sucrose synthase (Baxter and Duffus, 1973).

It has been suggested that the hydrolysis of sucrose results in hexose phosphates which in turn are converted to triose phosphates. A triosephosphate translocator may exist in the amyloplast membrane similar to that present in the chloroplast (Jenner, 1976; Duffus, 1979). Triose phosphates in the amyloplasts could then be incorporated into starch as in the chloroplast.

The enzymes of starch synthesis in storage tissue have been extensively studied but until intact amyloplasts can be isolated from cereal endosperms the location of the enzymes and the permeability of the amyloplast membrane to precursors of starch can only be supposition. As in the leaf, ADP glucose phosphorylase seems to be involved in the regulation of starch synthesis.

Starch degrading enzymes have been detected in immature cereal endosperms. They include α-amylase (Duffus, 1969), phosphorylase (Baxter and Duffus, 1973), α-glucosidase and debranching enzyme (Williams, 1976). Their role is unknown.
Fig 1.5 Hypothetical scheme for the conversion of sucrose to starch in the cereal endosperm.

1. UDP (ADP) dependant sucrose synthase
2. Invertase
3. Hexokinase
4. Fructo-kinase
5. Glucosephosphate isomerase
6. Phosphoglucomutase
7. ADP (UDP) glucose pyrophosphorylase
8. Nucleosidediphosphate kinase
9. Inorganic pyrophosphatase
10. ADP (UDP) glucose starch synthase
as starch granule hydrolysis in immature wheat endosperms was not observed (Duffus and Murdoch, 1979).

Starch has been found in the pericarps of immature wheat grains (Sandstedt, 1946; May and Buttrose, 1959), and in the cross cells of wheat and barley (Percival, 1921; MacGregor et al., 1972; Morrison, 1976). It seems likely that photosynthesis in the cross cells could be a source of metabolites for starch synthesis. However, little is known about the enzymes of carbohydrate metabolism in the pericarp.

The disappearance of pericarp starch granules during early development led MacGregor et al. (1972) to suggest that these may act as a temporary energy store for the growing kernel. In the absence of firm evidence however, the function, if any, of pericarp starch remains unknown.

1.6.1 Assimilate Supply to the Grain

The partitioning of photosynthate between the vegetative parts and the grain of cereals is a key indicator of crop productivity. After anthesis, the developing grain becomes the major sink for assimilates. The supply of assimilates to the developing grain from green tissues and their subsequent uptake and utilisation are points at which limits could affect grain yield. However, assimilate supply is not thought to be a limiting factor of crop production in normal conditions (Duffus, 1979; Cochrane and Duffus, 1981).

Carbon fixed by photosynthesis in the upper leaves, stem and
green parts of the ear moves to the developing caryopses where it is incorporated mainly into starch. Sucrose is thought to be the major carbon source for starch synthesis in storage tissues and is loaded into the veins of the leaf from the mesophyll or bundle sheath cells. Phloem loading has been reviewed by Geiger (1975) and Giaquinta (1983). The movement of sugar from the source cell to the phloem varies according to the species and may be symplastic or apoplastic or a combination of both. In wheat leaves the route is probably symplastic (Kuo et al., 1974). Translocation of sugar then takes place through the vascular tissue of the stem and rachis to the base of the grain. The phloem of the vascular bundle of the cereal caryopsis is continuous with that of the stem but the xylem is not. A region of xylem discontinuity exists at the junction of the rachilla and the base of the grain of wheat and barley (Zee and O'Brien, 1970a; Kirby and Rymer, 1975).

The import of assimilate and phloem unloading in seeds has recently been reviewed by Thorne (1985). Assimilate import in maize differs from that in barley and wheat in that assimilates are unloaded from the phloem into the pedicel which is part of a thin pericarp which surrounds the embryo and endosperm. From the symplast of the pedicel, the assimilates move to the placento-chalazal region apoplastically, from where they enter endosperm transfer cells (Felker and Shannon, 1980). In wheat and barley, the vascular bundle runs the entire length of the furrow and unloading can occur along its entire length (Frazier and Appalanaidu, 1965; Cochrane and Duffus, 1979,
Sucrose is transported into the endosperm without hydrolysis in wheat (Jenner, 1974, 1980; Donovan et al., 1983; Ho and Gifford, 1984) and in barley (Chevalier and Lingle, 1983). Transfer cells are present in the nucellar projection of barley (Cochrane and Duffus, 1980). These occur in situations where solutes move from the symplast to the apoplast. It is therefore likely that sucrose moves in the symplast from the phloem through the chalaza into the nucellar projection where it enters the apoplast.

The movement of assimilate into and through the endosperm is under debate. Jenner (1974) suggested that assimilates moved in the apoplast of the starchy endosperm of wheat but plasmodesmata are present between the starchy endosperm cells of barley and so the possibility of symplastic transport between cells cannot be excluded (Duffus and Cochrane, 1982).

As the wheat grain matures, sudanophilic bodies accumulate in the chalaza and the walls become adcrusted with lipids and polyphenolic substances (Zee and O'Brien, 1970b).

The chalaza of barley grains also contains polyphenolic material from fourteen days after anthesis but it was not possible to determine at which age symplastic movement of assimilates ceased (Cochrane, 1983). The deposition of lipid material in the chalaza was suggested to be responsible for the termination of wheat grain growth by blocking the route through the chalaza (Sofield et al., 1977). However, Jenner
(1982) established that cessation of starch synthesis in wheat could not be attributed to a reduction in sucrose supply.

1.6.2 Assimilate supply from the Glumes and Pericarp

The glumes of wheat, including the sterile glumes, the lemma and palea, can supply assimilates to the developing grain (Bremner and Rawson, 1972). The grain nearest to any one glume was usually the preferred sink for assimilates, which suggested to the authors that discrete vascular connections existed between the glumes and grains. Radioactive carbon fixed by glume photosynthesis was incorporated into grains (Carr and Wardlaw, 1965).

$^{14}$C-label from radioactive carbon dioxide fixed by caryopses minus their transparent layers has been recovered in endosperm starch of barley and wheat grains (Nutbeam, 1978). Assimilates must therefore move from the pericarp to the developing endosperm but the route taken is unknown.

One of the aims of the present work therefore is to examine the possible routes whereby pericarp photosynthates can enter the endosperm. The properties and functions of the immature pericarp will also be examined, in particular the fixation of carbon dioxide by both the transparent layer and cross cells of the pericarp. Little is known about the contribution made to grain filling by the pericarp. By comparison with a mutant barley which has no chlorophyll in its cross cells, an assessment of the contribution by the
green pericarp to the deposition of storage material in the endosperm can be made.
2. GROWTH CHARACTERISTICS OF BARLEYS

2.1 Introduction

In any study of the characteristics of cereal grain development a method of describing the stages of development is required. The simplest method is to describe grains by the number of days which has elapsed since anthesis. However, aging of the grains in purely chronological terms makes no allowance for variation in the rate of grain development which may arise as a consequence of changes in the environmental conditions. This technique is therefore invalid, especially where comparisons are to be made of plants grown at various locations and at different times of the year. Another method of assessing the stage of development of grains is therefore required.

Ideally the method to be used must be quick, simple and reproducible. It should not need large numbers of grains. On the basis of the above criteria, measurements of dry weight or other constituents of the grain cannot be used as these are destructive.

A carefully described and recorded sequence of changes in the morphological characteristics of grains has therefore been selected as the most convenient method for estimating grain or caryopsis 'age'. That is, measurements of caryopsis, endosperm and embryo size combined with the appearance of tissues can be used to describe the developmental stage of grains or caryopses quickly and simply.

The morphological features of the two-row barley cv. Midas
are shown in Table 1 (Agricultural Biochemistry Department, East of Scotland College of Agriculture, Edinburgh; 1977). Cv. Midas is a spring barley which is grown for its malting quality and can be grown all year round in a glasshouse. The pericarp, lemma and palea of the grain all contain chlorophyll until about 40 days after anthesis.

In the present work, glasshouse-grown grains of cv. Midas are 'aged' with reference to this table and described in terms of the number of days after anthesis which have elapsed. Thus, using this table all glasshouse-grown material may be aged with reference to field-grown material.

No such description of the grains of Albino lemma has been prepared. Albino lemma is a six-row barley and is a chemically-induced mutant produced by the Plant Breeding Institute, Cambridge. The pericarp, lemma and palea of the grain are albino with the exception of small green areas at the tips of the lemma and palea. However, the awns, sterile glumes and remainder of the plant contain chlorophyll and the plant grows well in a glasshouse after vernalisation.

The outer layers of the caryopsis of Albino lemma are anatomically identical to those of barley cv. Midas (Cochrane and Duffus, 1979). Small starch granules are found in the cells of the transparent layer of Albino lemma during early grain development.

Starch is also present in plastids in the cross cells until
<table>
<thead>
<tr>
<th>AGE</th>
<th>Days after anthesis</th>
<th>Size of embryo</th>
<th>Size and Colour of pericarp</th>
<th>Transparent layer</th>
<th>Endosperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.5 mm</td>
<td>Pale green</td>
<td>White</td>
<td>Small 'pearl'.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.5 mm</td>
<td>5 mm</td>
<td>Liquid with thin transparent sliver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Bright green</td>
<td>5 mm x 2 mm</td>
<td>6 mm</td>
<td>Endosperm thin, flat</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Pin tip</td>
<td>Bright green</td>
<td>6 mm x 2.5 mm</td>
<td>Slightly rounded and yellowish</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>.35 mm</td>
<td>Bright green rounded</td>
<td>7.5 mm x 3 mm</td>
<td>Well-rounded and firm, yellow</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.2 mm</td>
<td>Bright green but some yellowness</td>
<td>8 mm x 3.5 mm</td>
<td>Well-rounded. 7 mm. Firm yellow.</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>2.5 mm</td>
<td>Fused a little with palea</td>
<td>8 mm x 4 mm</td>
<td>Yellowish Green</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aleurone comes off with testa. Grain fat.</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>3 mm</td>
<td>Yellow, with green edges</td>
<td>8 mm x 4.5 mm</td>
<td>Hard rubbery</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>8 mm x 4.5 mm</td>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>8 mm x 3.5 mm</td>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 Morphological characteristics of barley cv. Midas grown in the field at Bush Estate, Midlothian in 1977 (Biochemistry Dept., Edinburgh School of Agriculture).
midway through grain development (Cochrane and Duffus, 1979). Any contribution to grain-filling by photosynthesis in the cross cells can therefore be investigated by reference to data obtained from this mutant. Since, under glasshouse conditions, Albino lemma has large well-filled grains, it may be that the mutant has some special adaptations that compensate for the lack of chlorophyll in the cross cells.

The work reported here was undertaken in order to understand more fully the developmental characteristics of Albino lemma and to enable its use as a control plant in the assessment of the photosynthetic contribution of the immature pericarp to grain-filling.

2.2 Methods

2.2.1 Plant Material

The six-row barley Hordeum vulgare L. var. hexastichum Albino lemma was grown from seed in pots of 180 mm diameter. Six plants per pot were grown in Levingtons' compost at the Crop Production Glasshouse Unit, Bush Estate, Midlothian until a few days before anthesis when they were transferred to the Department of Agricultural Biochemistry Glasshouse at Kings Buildings, Edinburgh. Natural daylength was extended to 20 h with 400 W mercury vapour lamps and the mean daily temperature was 18°C in the Biochemistry glasshouse. Insect and fungal infections were minimised by regular spraying with Pirimor (I.C.I.) and Milgo (I.C.I.). The plants were fed once a week with Solufeed nutrient solution (I.C.I.).
The two-row barley *Hordeum vulgare* L. var. *distichum* cv. *Midas* was also grown as described above.

### 2.2.2 Glasshouse Sampling

Ears of *Albino lemma* were cut from glasshouse-grown plants and 5 grains removed from the middle of each of 3 alternate rows (the rows with the biggest grains). The lemma and palea were dissected from the caryopsis and the length and width of the caryopsis were noted. After removal of the pericarp, testa and nucellar epidermis from the caryopsis, the lengths and widths of the endosperm and embryo were noted. The age of each ear of *Albino lemma* was estimated by aging an ear of cv. *Midas* which reached anthesis on the same day as the *Albino lemma* ear. The cv. *Midas* ear was aged with reference to Table 2.1.

### 2.2.3 Fresh and Dry Weights of Grains

Five grains were removed from the middle of 3 alternate rows of 4 ears of *Albino lemma*. The grains were placed on moist filter paper, pooled and 30 grains randomly sampled. The awns were cut off at the top of the lemma and the rachillas removed. Ten grains were placed, in each of 3 glass vials and the weight of the grains noted. The vials were placed in an oven at 70°C and dried until constant weight was achieved. Grains of cv. *Midas* were sampled as above.

### 2.2.4 Fresh and Dry Weights of Caryopses

Grains of *Albino lemma* were sampled as described in Section 2.2.2 and the husks removed. Ten caryopses were placed in
each of 3 glass vials and the fresh weight of the caryopses noted. The vials were placed in an oven at 70°C and dried until constant weight was achieved. Caryopses of cv. Midas were sampled as above.

2.3 Results

2.3.1 Morphological Changes in the Caryopses of Albino lemma and cv. Midas

As the caryopsis of Albino lemma matured it expanded lengthwise until it reached a maximum of 8.3 mm at about 35 days after anthesis (Table 2.2). The width increased until about 40 days after anthesis when it was 5.0 mm. The caryopsis at this stage was large, plump and white (Fig. 2.2c). At about 50 days after anthesis the husk and caryopsis are fused and cannot be separated easily. Harvest ripeness was reached 60 days after anthesis when the grain was shrivelled and golden in colour (Fig. 2.2d).

An ear of Albino lemma aged 25 days after anthesis is shown in Fig. 2.1. The stem, awns and the tips of lemma and palea were green at this age (Fig. 2.1, 2.4) but by 40 days after anthesis the green colour began to disappear. The lack of chlorophyll in the pericarp is apparent from Fig. 2.2 as was the increase in size of the caryopsis.

The endosperm and embryo of Albino lemma also increased in size until 40 - 47 days after anthesis when they reached maximum size. At this age the endosperm cannot be dissected free of
<table>
<thead>
<tr>
<th>Age (days after anthesis)</th>
<th>Size of caryopsis length x width (mm)</th>
<th>Size of endosperm length x width (mm)</th>
<th>Size of embryo length x width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8 x 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.0 x 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.2 x 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>7.5 x 2.3</td>
<td>5.0 x 1.5</td>
<td>pintip</td>
</tr>
<tr>
<td>25</td>
<td>8.0 x 2.9</td>
<td>6.5 x 2.0</td>
<td>0.8 x 0.5</td>
</tr>
<tr>
<td>28</td>
<td>8.0 x 3.0</td>
<td>6.5 x 2.7</td>
<td>1.1 x 1.0</td>
</tr>
<tr>
<td>33</td>
<td>8.0 x 3.5</td>
<td>6.6 x 2.7</td>
<td>1.8 x 1.5</td>
</tr>
<tr>
<td>35</td>
<td>8.3 x 3.8</td>
<td>7.0 x 3.0</td>
<td>2.1 x 1.5</td>
</tr>
<tr>
<td>40</td>
<td>8.0 x 5.0</td>
<td>7.2 x 3.8</td>
<td>2.8 x 2.0</td>
</tr>
<tr>
<td>47</td>
<td>8.0 x 4.0</td>
<td>7.7 x 3.6*</td>
<td>2.7 x 2.0</td>
</tr>
<tr>
<td>50</td>
<td>8.0 x 4.0+</td>
<td>7.0 x 3.6*</td>
<td>2.5 x 2.0</td>
</tr>
<tr>
<td>55</td>
<td>7.8 x 3.5+</td>
<td>6.1 x 3.2*</td>
<td>2.1 x 2.0</td>
</tr>
<tr>
<td>60</td>
<td>7.0 x 3.5+</td>
<td>6.1 x 3.0*</td>
<td>2.0 x 2.0</td>
</tr>
</tbody>
</table>

Table 2.2 Morphological changes in the caryopsis of barley Albino lemma.

+ caryopsis fused to husk
* endosperm fused to testa
Fig. 2.1 Ear of *Albino lemma* aged 25 days after anthesis.

Fig. 2.2 Caryopses of *Albino lemma* aged a) 15 days b) 25 days c) 40 days d) 60 days after anthesis (grain).

Fig. 2.3 Transverse section of a caryopsis of *Albino lemma* aged 25 days after anthesis.

Fig. 2.4 Grain of *Albino lemma* aged 25 days after anthesis.

Fig. 2.5 Ear of cv. Midas aged 25 days after anthesis.

Fig. 2.6 Caryopses of cv. Midas aged a) 25 days b) 40 days c) 60 days after anthesis (grain).

Fig. 2.7 Caryopsis of cv. Midas minus the transparent layer aged 25 days after anthesis.
the testa. A noticeable feature of the endosperm was the large endosperm cavity (Fig. 2.3).

The barley cv. Midas contained chlorophyll in its cross cells until about 40 days after anthesis (Fig. 2.6). The lemma and palea of the grain were also green (Fig. 2.5). Grains of cv. Midas were small and reached maximum size, 8.0 x 4.5 mm, 40 days after anthesis (Table 2.1). The endosperm reached maximum size 35 days after anthesis at which stage the testa and endosperm were difficult to separate. At maturity the grain of cv. Midas was golden yellow and small (Fig. 2.6c).

2.3.1 Fresh and Dry Weights of Grains of Albino lemma and cv. Midas

The changes in fresh and dry weights of grains of Albino lemma are shown in Fig. 2.8. The fresh weight increased gradually from anthesis to about 25 days after anthesis where the increase was more rapid. At 40 days after anthesis maximum fresh weight was reached (730 ± 70 mg per 10 grains) and thereafter declined quite rapidly to 360 ± 15 mg per 10 grains at harvest ripeness or maturity.

Similarly, the dry weight of the grains increased slowly until 25 days after anthesis when the increase became more rapid. At 47 days after anthesis the dry weight reached a maximum of 330 ± 30 mg per 10 grains which was also the dry weight of grains at harvest ripeness.

The water content of the grains was also estimated. This was
Fig. 2.8 Fresh and dry weights of grains of *Albino lemma*.

- △ fresh weight
- ▲ dry weight
- • % water content

error bars represent standard deviations

Fig. 2.9 Fresh and dry weights of grains of cv. Midas grown in a glasshouse (M.P. Cochrane, 1983).

Fig. 2.10 Fresh and dry weights of caryopses of *Albino lemma*.

Fig. 2.11 Dry weight of caryopses of cv. Midas grown in a glasshouse (M.P. Cochrane).
about 70% for the first 30 days of the grain-filling period and then declined as the grain matured. At maturity the water content was 11% of the total grain weight.

The changes in the dry weight of grains of cv. Midas are shown in Fig. 2.9. Dry weight increased rapidly at 11 days after anthesis and reached a maximum of $419 \pm 10$ mg per 10 grains 50 days after anthesis. Thereafter the dry weight of the grains declined to $366 \pm 7$ mg per 10 grains at 53 days after anthesis when the grains were at harvest ripeness. The gain in dry weight of cv. Midas grains started at an earlier stage than with Albino lemma and not until about 40 days after anthesis did the grains of the two barleys reach similar dry weight.

The fresh weights of cv. Midas grains are shown in Fig. 2.9. From 11 days after anthesis the fresh weight increased rapidly to a maximum of about 700 mg per 10 grains at 29 to 39 days after anthesis, thereafter declining to 550 mg per 10 grains at 53 days after anthesis which was harvest ripeness.

2.3.3 Fresh and Dry Weights of Caryopses of Albino lemma and cv. Midas

The changes in the fresh and dry weights of caryopses of Albino lemma were recorded until about 42 days after anthesis when the husk started to fuse to the caryopsis. At this stage it was not possible to remove the husk without damaging the caryopsis.

The fresh weight of the caryopses increased rapidly after 10 days and was $545 \pm 50$ mg per 10 caryopses by 42 days after anthesis.
Fig. 2.10. The dry weight increased slowly until about 30 days after anthesis and thereafter was rapid until it reached 225 ± 20 mg per 10 caryopses at 42 days. Between 30 and 36 days after anthesis the dry weight increased dramatically. This rapid increase was not observed with grains of Albino lemma. This difference might be explained by independent variation in the dry weight of the husk. The dry weight changes of caryopses of cv. Midas were also recorded (Fig. 2.11). At 40 days after anthesis the dry weight of 10 caryopses was 354 mg. After this age the husk becomes fused to the caryopses and cannot be removed. As with Albino lemma the increase in dry weight was slow until 27 days after anthesis.

2.4 Discussion

A description of morphological changes accompanying grain and caryopsis development is a simple and quick method for aging grains prior to use. The age of an ear can therefore be ascertained by sampling and aging a few grains from the middle of the ear. If intact ears are needed, the size of the grains can be assessed without removing them from the rachis and a more accurate estimate of age made when the experimental procedure is completed. With this method, grains at the same stage of development can be selected and compared with others used in previous experiments.

In the following chapters the biochemical and physiological characteristics of the pericarp of cv. Midas are compared with those of Albino lemma. The absence of chlorophyll in the cross cells of Albino lemma allows the role of pericarp photo-
synthesis in grain filling to be studied. However, little is known about the mutant and it may have genetic lesions other than the absence of chlorophyll in certain tissues. Also, Albino lemma is a six-row barley whereas as cv. Midas is two-row. A two-row albino mutant was not available and whilst six-row 'normal' barleys are grown, cv. Midas was chosen as it is a popular spring barley grown for its good malting qualities.

Albino lemma was not grown in the field as it has long, brittle stems which make it susceptible to lodging. It also requires vernalisation and was therefore grown only in the winter and spring months whereas cv. Midas grew well throughout the year.

The dry weights at maturity of Albino lemma and cv. Midas were not significantly different but as described previously, cv. Midas has unusually small grains.

Two-row cultivars grown in the field with thousand grain weights at maturity of 49g (cv. Koru), 43g (cv. Triumph) and 39g (cv. Golden Promise) have been recorded (Cochrane and Duffus, 1983). Of these cultivars only cv. Koru has a significantly higher grain dry weight at maturity than Albino lemma. It seems that Albino lemma at least when grown in the glasshouse has a dry weight at harvest ripeness which is within the range of most two-row barleys grown under field conditions. It is not possible to state however, from the results, whether or not the lack of chlorophyll in the outer layers of the grain has a deleterious effect on the final dry weight of Albino lemma - in particular under field conditions.
During early grain development, the dry weight increase of cv. Midas grains is rapid. At 27 to 30 days after anthesis when the chlorophyll content of the pericarp is at a maximum (Duffus and Rosie, 1973), the dry weight of cv. Midas grains is twice that of Albino lemma grains. It could be that, during this period, grain photosynthates are used for endosperm starch synthesis in addition to the assimilates supplied by the leaves, stem, rachis and sterile glumes. Albino lemma, which has no chlorophyll in its pericarp but some in the lemma and palea, would have fewer sources of assimilates, thus resulting in a slower weight gain. However, comparison of the dry weights of caryopses of the two barley's shows that they are remarkably similar.

It seems likely that the differences in grain dry weight during early development can be attributed to weight gains in the lemma and palea of cv. Midas. Whether the increased weight is due to starch or some other product synthesised as a consequence of increased photosynthesis or partitioning is as yet unknown.

The observation that Albino lemma stems are very brittle and prone to breaking under field conditions suggests that the stem structure is impaired, possibly as a result of a reduction in the synthesis of stem carbohydrate reserves. Thus it may be that in 'normal' cultivars the role of the pericarp may be to prevent the depletion of stem reserves by generating a supply of photosynthetic assimilates.

Any role for pericarp photosynthesis in grain filling must take note that, despite the absence of chlorophyll in the pericarp, the grains of Albino lemma are large and well-filled with con-
Considerable starch deposits present in the transparent layer and cross cells of the pericarp.
3. CARBON DIOXIDE FIXATION BY DETACHED CARYOPSES

3.1 Introduction

Many investigations into cereal production have demonstrated the importance of ear photosynthesis to yield (Archbold, 1942; Birecka et al., 1964; Thorne, 1966). The major source of assimilates for grain filling is thought to be photosynthesis by the ear, stem and flag leaf after anthesis although it has been suggested that material stored in the stem before anthesis makes a significant contribution to final grain yield (Biscoe et al., 1976; Bidinger et al., 1977; Stoy, 1979, 1980). Whilst estimates of the contribution made by ear photosynthesis to total photosynthetic activity depend on factors such as the cultivar, the presence or absence of awns, the environment, and the technique used, it has been shown that all the green tissues of the ear are capable of photosynthesis and thus could contribute to yield (Carr and Wardlaw, 1965; Thorne, 1965; Evans and Rawson, 1970; Bremner and Rawson, 1972). These tissues are therefore, potential sources of assimilates for grain filling.

Carr and Wardlaw (1965) found that the glumes and caryopses of wheat were capable of photosynthesis. Excised caryopses could also fix externally-supplied $^{14}\text{CO}_2$ (Evans and Rawson, 1970). This was confirmed by Radley (1976) and Wirth et al. (1977). Caryopses of barley (Nutbeam and Duffus, 1976) and Italian ryegrass (Ong et al., 1978) were also capable of $^{14}\text{CO}_2$ fixation.

$^{14}\text{CO}_2$ fixation by excised caryopses of wheat with and without the transparent layer of the pericarp was studied by Radley (1976).
She noted that more $^{14}\text{C}$ was recovered from caryopses minus the transparent layer and attributed this to the impermeability of the transparent layer to CO$_2$. However, Cochrane and Duffus (1979) and Gifford and Bremner (1981a) found stomata in the pericarp epidermis which, if functional, would allow the passage of CO$_2$ to the cross cells of the pericarp. Additionally, although the pericarp epidermis has a cuticular layer, it is likely that it may be permeable to CO$_2$ (Cochrane and Duffus, 1979).

It was briefly noted by Nutbeam (1978) that a $^{14}\text{C}$-labelled insoluble fraction was present in the endosperm of barley caryopses cv. Julia following incubation of caryopses minus the transparent layer. Otherwise little is known of the contribution to grain filling of layers which surround the immature endosperm. The objective of the present work is therefore to investigate the possible role of these layers in the fixation of externally supplied CO$_2$ by describing the distribution of $^{14}\text{C}$ between tissues of excised intact caryopses following incubation in $^{14}\text{CO}_2$. The fixation of CO$_2$ by the transparent layer of the pericarp is of particular interest as small starch granules are present in this tissue during early grain development (Percival, 1921; Krauss, 1933) but disappear as the grain matures (MacGregor et al., 1972). Little is known about the source of metabolites for starch synthesis or the properties of this tissue.

3.2 Methods

3.2.1 Plant Material

The barleys cv. Midas and Albino lemma and the wheat, Triticum
Aestivum cv. Sicco were grown as described in Section 2.2.1. Caryopses of cv. Midas and Albino lemma were aged as described in Sections 2.2 and 2.3 and caryopses of cv. Sicco were aged using a description of the growth characteristics of field grown wheat cv. Sentry harvested in 1981 (A.D. Cronshaw, personal communication).

3.2.2 Fixation of Radioactive Carbon Dioxide (\(^{14}\text{CO}_2\)) by Detached Caryopses

A modification of the method described by Nutbeam (1978) was used. The sterile glumes, lemma and palea were dissected from 4 grains and the caryopses placed on filter paper discs previously soaked in 330 mM sorbitol, 50 mM Tricine-KOH, pH 7.5. In certain experiments the transparent layer of the pericarp was removed. The detached caryopses on moist discs were placed in an airtight perspex chamber (volume 11 cm\(^3\)) and 0.02 cm\(^3\) of 17 mM sodium (\(^{14}\text{C}\)) carbonate (specific activity 58.4 mCi mmol\(^{-1}\)) was injected through a rubber seal into a well containing 0.15 cm\(^3\) of 13 M lactic acid. The final concentration of CO\(_2\) in the chamber was 0.1% by volume.

The chamber was illuminated with a 1000 W tungsten halogen lamp (photon flux density in the chamber was 710 \(\mu\text{mol m}^{-2}\text{s}^{-1}\)) for varying periods of time. A perspex box (dimensions 250 x 250 x 60 mm) with cold water circulating through it was positioned between the lamp and the chamber to maintain the chamber at 21°C. The light-dependence of \(^{14}\text{CO}_2\) fixation was investigated by covering the chamber with aluminium foil. The conditions
for the dark experiments were as described for investigations in the light.

3.2.3 Extraction of $^{14}$C-labelled Material from Caryopses

Following incubation with $^{14}$CO$_2$, the caryopses were rinsed quickly in distilled water and the transparent layer dissected from the caryopsis using fine tweezers. The cross cells, testa and nucellar epidermis (inner layers) were removed together as these tissues are difficult to separate from caryopses aged 25 days after anthesis. Similarly, the embryo was extracted with the endosperm as the embryo is very small at this stage of caryopsis development. The tissues were homogenised in 80% (v/v) ethanol at 70°C with hand-held all-glass tissue grinders. Each homogenate was washed twice with two aliquots of hot ethanol and the washings plus ethanol-insoluble material were centrifuged for 2 min at 700 g. The supernatant was made up to 10 cm$^3$ and two 0.1 cm$^3$ samples were mixed with 15 cm$^3$ of a Triton X-100/toluene scintillation cocktail (660 cm$^3$ of scintillation grade toluene, 330 cm$^3$ of Triton X-100, 4 g of 2,5-diphenyloxazole) and counted for 10 min in a Beckman LS100C liquid scintillation spectrometer.

The ethanol-insoluble residue was mixed with 5 cm$^3$ of distilled water and left for 24 h at room temperature. After centrifugation at 700 g for 2 min, two 0.1 cm$^3$ samples of the supernatant were counted for $^{14}$C-activity. A modification of the method of Pucher et al. (1948) was used to hydrolyse the water-insoluble material. The residue was washed twice with 4 cm$^3$ of distilled water, 2 cm$^3$ of 2M HCL was added and the solution heated for about
2 h at 100 C. A drop of the solution was added to I₂/KI solution to ensure that starch had been hydrolysed. Samples were counted for ¹⁴C-activity as before. All measurements of radioactivity were corrected to disintegrations per min (dpm) using quench curves.

Quench curves were prepared by adding small volumes of quenching agents (unlabelled tissue extracts) to known amounts of a ¹⁴C-labelled compound. The ¹⁴C standards were counted before and after the addition of the quenching agents and the efficiency of counting was plotted against the External Standard Ratio (ESR) of the standards which was computed by the scintillation counter. The efficiency of counting experimental samples was found using their ESR values. Unlabelled tissues were extracted and counted for ¹⁴C-activity as described above and the values subtracted from those of radioactive tissues thus giving an estimate of background radiation.

3.3 Results

3.3.1 ¹⁴C Distribution in Caryopses of cv. Midas

The distribution of ¹⁴C between tissues of intact barley caryopses cv. Midas after incubation with ¹⁴CO₂ in the light and dark is shown in Table 3.1. After exposure to ¹⁴CO₂ for 2.5 min in the light, 670 dpm were recovered per caryopsis. The ¹⁴C was present only in the combined ethanol and water-soluble fractions of the three tissues. After exposure to ¹⁴CO₂ for 5 min, the ethanol and water-insoluble fractions of the inner layers was also labelled. The majority of the ¹⁴C (4390 dpm per caryopsis) was found in the soluble fraction of the inner layers. The
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Duration of 14CO₂ incubation (min)</th>
<th>Light (L) or Dark (D)</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>2.5</td>
<td>L</td>
<td>130 (19.4)</td>
<td>0 (0)</td>
<td>130 (19.4)</td>
</tr>
<tr>
<td>Inner layers</td>
<td></td>
<td></td>
<td>520 (77.6)</td>
<td>0 (0)</td>
<td>520 (77.6)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td></td>
<td>20 (3.0)</td>
<td>0 (0)</td>
<td>20 (3.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>670 (100)</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>5</td>
<td>L</td>
<td>740 (16.9)</td>
<td>0 (0)</td>
<td>740 (16.9)</td>
</tr>
<tr>
<td>Inner layers</td>
<td></td>
<td></td>
<td>3,190 (72.7)</td>
<td>230 (5.2)</td>
<td>3,420 (77.9)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td></td>
<td>230 (5.2)</td>
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<td>230 (5.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4,390 (100)</td>
</tr>
<tr>
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<td>10</td>
<td>L</td>
<td>2,680 + 410 (19.4)</td>
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<td>2,680 (19.4)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>10,230 + 230 (74.0)</td>
<td>250 + 250 (1.8)</td>
<td>10,480 (75.8)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td></td>
<td>660 + 80 (4.8)</td>
<td>0 (0)</td>
<td>660 (4.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13,820 (100)</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>15</td>
<td>L</td>
<td>5,540 + 2,660 (6.4)</td>
<td>200 + 100 (0.2)</td>
<td>5,740 (5.6)</td>
</tr>
<tr>
<td>Inner layer</td>
<td></td>
<td></td>
<td>80,370 + 3,730 (78.7)</td>
<td>2250 + 820 (2.2)</td>
<td>82,620 (80.9)</td>
</tr>
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<td></td>
<td></td>
<td>12,660 + 950 (12.4)</td>
<td>100 + 200 (0.1)</td>
<td>12,760 (12.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>102,120 (100)</td>
</tr>
<tr>
<td>Transparent layer</td>
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<td>D</td>
<td>150 (100)</td>
<td>0 (0)</td>
<td>150 (100)</td>
</tr>
<tr>
<td>Inner layers</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>150 (100)</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>15</td>
<td>D</td>
<td>240 + 120 (28.8)</td>
<td>50 + 30 (5.9)</td>
<td>290 (34.5)</td>
</tr>
<tr>
<td>Inner layers</td>
<td></td>
<td></td>
<td>200 + 30 (23.8)</td>
<td>50 + 10 (5.9)</td>
<td>250 (29.7)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td></td>
<td>210 + 100 (25.0)</td>
<td>90 + 20 (10.8)</td>
<td>300 (35.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>840 (100)</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>15</td>
<td>L</td>
<td>3,040 + 350 (97.8)</td>
<td>70 + 10 (2.2)</td>
<td>3,110 (100)</td>
</tr>
</tbody>
</table>

Table 3.1 14C in caryopses of cv. Midas aged 25 days after anthesis after incubation with 14CO₂. Values are averages of 3 separate determinations ± s.d. except 1 which are averages of 2 separate determinations.
total $^{14}$C recovered per caryopsis increased to 13820 dpm after incubation of the caryopses with $^{14}$CO$_2$ for 10 min. Similarly, the majority of the $^{14}$C was recovered from the soluble fraction of the inner layers (74.0%). Labelling in the insoluble fraction was not significant. The percentages of the total $^{14}$C recovered per caryopsis were between 3.0 and 5.2% for the soluble fractions of the endosperm and 16.8 to 19.4% for the transparent layer after 2.5 to 10 min in $^{14}$CO$_2$.

Following exposure to $^{14}$CO$_2$ for 15 min in the light the total $^{14}$C recovered per caryopsis was 102120 dpm. The majority of the $^{14}$C was found in the soluble fraction of the inner layers (78.7%) and the endosperm (12.4%). The percentage of the total $^{14}$C in the soluble fraction of the transparent layer was considerably less than that observed for the shorter times. All three tissues contained $^{14}$C in their insoluble fractions but that in the endosperm was not significant. Some of the standard deviations are large and therefore the values are not significant.

Intact caryopses were also exposed to $^{14}$CO$_2$ for 10 and 15 min in darkness. Little $^{14}$C was recovered after 10 min exposure but 840 dpm per caryopsis were found after 15 min. The soluble and insoluble fractions of all three tissues contained $^{14}$C although that found in the insoluble fractions was very small.

The transparent layers were dissected from caryopses and exposed to $^{14}$CO$_2$ for 15 min in the light. Most of the $^{14}$C was found in the soluble fraction (97.8%) but some $^{14}$C was present in the
insoluble fractions. The total $^{14}$C recovered (3110 dpm) was less than that in the tissue when attached to the caryopsis (5740 dpm).

Caryopses of barley cv. Midas minus their transparent layers were also exposed to $^{14}$CO$_2$ in both the light and dark (Table 3.2). After 10 min in the light the total $^{14}$C per caryopsis was 96360 dpm of which 87.9% was recovered from the inner layers and 12.1% from the endosperm. The majority of the label was in the soluble fraction of the inner layers (82.9%) with 5.0% and 0.4% in the insoluble fractions of the inner layers and endosperm respectively.

Almost three times as much $^{14}$C was recovered from the caryopses after a 15 min exposure to $^{14}$CO$_2$. Again the majority of the $^{14}$C was in the soluble fraction of the inner layers (85.2%). The distribution of $^{14}$C between the tissue fractions was similar to that found after exposure to $^{14}$CO$_2$ for 10 min.

After incubation for 10 min in darkness the caryopses contained 4970 dpm per caryopsis. $^{14}$C was found only in the soluble fractions of the tissues.

3.3.2 $^{14}$C Distribution in Caryopses of Albino lemma

After incubation with $^{14}$CO$_2$ in both the light and dark, caryopses of barley Albino lemma were dissected and the distribution of $^{14}$C between tissues was estimated (Table 3.3). Intact caryopses contained 6280 dpm per caryopsis after exposure to $^{14}$CO$_2$ for 10 min in the light. The majority of the $^{14}$C was recovered
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Duration of $^{14}$CO$_2$ incubation (min)</th>
<th>Light (L) or dpm/caryopsis</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner layers</td>
<td>10</td>
<td>79,890 ± 100 (82.9)</td>
<td>4,820+1,920 (5.0)</td>
<td>84,710 (87.9)</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td>11,270 ± 2590 (11.7)</td>
<td>380+290 (0.4)</td>
<td>11,650 (12.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96,360 (100)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>15</td>
<td>225,000 ± 5490 (85.2)</td>
<td>1,664+7,130 (6.3)</td>
<td>241,640 (91.5)</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td>21,920 ± 1130 (8.3)</td>
<td>530+260 (0.2)</td>
<td>22,450 (8.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>264,090 (100)</td>
</tr>
<tr>
<td>Inner layers</td>
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<td>3,380 (68.1)</td>
<td>0</td>
<td>(0)</td>
<td>3,380 (68.1)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td></td>
<td>1,590 (31.9)</td>
<td>0</td>
<td>(0)</td>
<td>1,590 (31.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4,970 (100)</td>
</tr>
</tbody>
</table>

Table 3.2 $^{14}$C in caryopses of cv. Midas aged 25 days after anthesis after incubation with $^{14}$CO$_2$. Values are averages of 3 separate determinations ± s.d. except 1 which is the average of 2 separate determinations.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Duration of $^{14}$CO$_2$ incubation (min)</th>
<th>Light (L)</th>
<th>Dark (D)</th>
<th>dpm/caryopsis (% of total $^{14}$C recovered)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>10</td>
<td>L</td>
<td>D</td>
<td>3,310 (52.7)</td>
<td>3,310</td>
</tr>
<tr>
<td>Inner layers</td>
<td>10</td>
<td>D</td>
<td>1,420 (43.9)</td>
<td>1,420</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>10</td>
<td>D</td>
<td>1,150 (33.5)</td>
<td>1,150</td>
<td></td>
</tr>
<tr>
<td>Inner layers</td>
<td>15</td>
<td>D</td>
<td>3,550 (46.7)</td>
<td>3,550</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>15</td>
<td>D</td>
<td>1,850 (24.3)</td>
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<td></td>
</tr>
<tr>
<td>Inner layers</td>
<td>10</td>
<td>L</td>
<td>10,890 (40.6)</td>
<td>11,180</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
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<td>D</td>
<td>2,200 (29.0)</td>
<td>2,200</td>
<td></td>
</tr>
<tr>
<td>Inner layers</td>
<td>20</td>
<td>L</td>
<td>8,610 (28.2)</td>
<td>8,610</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>20</td>
<td>D</td>
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<td>21,900</td>
<td></td>
</tr>
<tr>
<td>Inner layers</td>
<td>15</td>
<td>D</td>
<td>4,190 (31.9)</td>
<td>4,230</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>15</td>
<td>D</td>
<td>8,890 (67.6)</td>
<td>8,920</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3 $^{14}$C in caryopses of Albino lemma aged 25 days after anthesis after incubation with $^{14}$CO$_2$. Values are averages of 3 separate determinations ± s.d., except 1 which is the average of 2 separate determinations.
from the soluble fraction of the transparent layer (52.7%) with only 16.9% in the inner layers. No $^{14}$C was found in the insoluble fractions.

After 10 and 15 min in the dark with $^{14}$CO$_2$ the caryopses contained 3240 dpm and 7600 dpm respectively. The distribution of $^{14}$C between the tissues was similar with the soluble fractions of the transparent layers containing almost half the total $^{14}$C recovered. No $^{14}$C was found in the insoluble fractions.

Caryopses of Albino lemma minus the transparent layer were incubated for 10 min in the light with $^{14}$CO$_2$. Of the total $^{14}$C recovered (26820 dpm per caryopsis), 40.6% was found to be in the soluble fraction of the inner layers and 58.3% in the endosperm. The insoluble fraction of the inner layers contained 290 dpm or 1.1% of the total $^{14}$C. No $^{14}$C was recovered from the insoluble fraction of the endosperm.

After 20 min in the light the total $^{14}$C per caryopsis was 30510 dpm. Most of the $^{14}$C was in the soluble fraction of the endosperm (71.4%). No $^{14}$C was found in the insoluble fraction of the inner layers but 120 dpm (0.4%) were present in the endosperm.

Caryopses minus the transparent layer were also incubated for 15 min in darkness. The total $^{14}$C per caryopses was 13150 dpm of which 67.6% was in the soluble fraction of the endosperm. Both the insoluble fractions contained a small amount of $^{14}$C.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>dpm/caryopsis (% of total $^{14}$C recovered)</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble</td>
<td>Insoluble</td>
<td></td>
</tr>
<tr>
<td>Transparent layer</td>
<td>53,840 ± 4,270 (46.96)</td>
<td>160 ± 50</td>
<td>54,000 (47.10)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>47,080 ± 1,180 (41.06)</td>
<td>1,970 ± 160 (1.72)</td>
<td>49,050 (42.78)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>11,570 ± 400 (10.09)</td>
<td>40 ± 10</td>
<td>11,610 (10.12)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>86,800 ± 4,640 (45.4)</td>
<td>70,590 ± 14,780 (36.9)</td>
<td>157,390 (82.3)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>32,270 ± 650 (16.9)</td>
<td>1,590 ± 180 (0.8)</td>
<td>33,860 (17.7)</td>
</tr>
</tbody>
</table>

Table 3.4 $^{14}$C in caryopses of wheat cv. Sicco aged 25 days after anthesis after incubation with $^{14}$CO$_2$ for 15 min in the light. Values are averages of 3 separate determinations ± s.d.
Intact caryopses of wheat cv. Sicco were incubated with $^{14}\text{C}\text{CO}_2$ for 15 min in the light. The total $^{14}\text{C}$ recovered per caryopsis was 114660 dpm and all tissue fractions were $^{14}\text{C}$-labelled (Table 3.4). The soluble fraction of the transparent layer contained more $^{14}\text{C}$ than either the inner layers or the endosperm. However, the insoluble fraction of the inner layers contained 1.72% of the total $^{14}\text{C}$ which was considerably more than that recovered from the transparent layer (0.14%) and the endosperm (0.03%).

Caryopses of cv. Sicco minus the transparent layer were also exposed to $^{14}\text{CO}_2$ for 15 min in the light. Of the total $^{14}\text{C}$ recovered per caryopsis (191250 dpm), 36.9% or 70590 dpm were found in the insoluble fraction of the inner layers and 0.8% in the endosperm. The soluble fraction of the inner layers was also heavily labelled (86800 dpm).

3.4 Discussion

Carbon dioxide fixation by two barley varieties and one wheat cultivar was investigated using $^{14}\text{C}$-labelling. Previously, photosynthesis by grains and caryopses of cereals has been studied using both isotope labelling and gas exchange measurements (Evans and Rawson, 1970; Radley, 1976; Nutbeam and Duffus, 1978; Wirth et al., 1977). The origin of the CO$_2$ for pericarp photosynthesis is unknown. It may be derived from atmospheric CO$_2$ or from CO$_2$ respired by tissues of the caryopsis. These experiments investigated the fixation of externally-supplied CO$_2$
by excised cereals caryopses. After incubation in both the light or dark with $^{14}$CO$_2$, radioactivity was detected in the transparent layer, inner layers, and endosperms plus embryos. This confirms that immature cereal caryopses have the ability to fix externally-supplied CO$_2$ and is agreement with work on wheat and barley by Evans and Rawson (1972), Radley (1976), Nutbeam (1978) and Wirth et al. (1978).

Caryopses aged 25 days after anthesis were used since at this time, the chlorophyll content of barley cross cells is maximal (Duffus and Rosie, 1973). At the same time the grain growth rate is also maximal (Fig. 2.9). In vivo, at this age, the caryopsis of wheat is loosely surrounded by glumes which have been reported to reduce the incident photon flux density by up to 78% (Evans and Rawson, 1970). It is likely that similar observations could be made for barley. However, at this reduced photon flux density the photosynthetic rate of the wheat caryopsis was about 90% of the rate measured at saturating photon flux density. The photon flux density used in these experiments ensured that light was not a limiting factor.

The total $^{14}$C recovered per caryopsis of cv. Midas minus the transparent layer was consistently greater than that recovered from intact caryopses. Albino lemma and cv. Sicco caryopses also exhibited this difference. Previously Radley (1976) had observed this effect using wheat caryopses and attributed the increase in $^{14}$CO$_2$ fixation by the cross cells to the impermeability of the transparent layer to CO$_2$. In contrast, Cochrane and Duffus (1979) suggested that the transparent layer is perm-
eable to CO$_2$ but may not readily be permeable to O$_2$ produced by cross cell photosynthesis. If this is indeed the case, the O$_2$ would build up in the airspace between the transparent layer and cross cells thus inhibiting RuBP carboxylase activity. However, the predominating carboxylase in barley cross cells is PEP carboxylase which is not inhibited by O$_2$ (Nutbeam and Duffus, 1976, Chapter 6). The increase in CO$_2$ fixation upon removal of the transparent layer from Albino lemma caryopses supports Radley's hypothesis that the transparent layer acts as a barrier to CO$_2$ movement. Removal of this tissue would allow CO$_2$ to be freely available to the cross cells.

When the transparent layer is present, atmospheric CO$_2$ could reach the cross cells by diffusion through the cuticle or the stomata on the pericarp epidermis. Stomata have been found at the brush end of barley caryopses on the flanks of the crease (Cochrane and Duffus, 1979), and in the pericarp epidermis of wheat (Bradbury et al., 1956a; Gifford and Bremner, 1981a). It is not known whether these stomata are functional. However, both open and shut stomata have been observed in the pericarp of the barley cv. Midas (L. Craig, personal communication).

The presence of $^{14}$C-label in the transparent layer suggests either that $^{14}$CO$_2$ can be fixed directly by this tissue and/or that $^{14}$CO$_2$ may move into the cross cells where it is incorporated into photosynthetic assimilates which may then be translocated to the transparent layer.

As the total $^{14}$C in the transparent layer was greater when the tissue was attached to the caryopsis it is possible that the
cross cells do supply the transparent layer with assimilates. However, the transparent layer could have been damaged when dissected from the caryopsis. The transparent layer of cv. Sicco contained about eight times as much $^{14}$C as cv. Midas which suggests that the wheat tissue has higher carboxylase activity.

These experiments also show that the caryopses of cv. Midas and cv. Sicco have the ability to translocate $^{14}$C-labelled assimilates to the endosperm which is in agreement with the preliminary work of Nutbeam (1978) and Radley (1976). There is some evidence to suggest that assimilates may be incorporated into insoluble material which is probably mainly starch with some cell wall material. However, it is only significant (and the amounts are very small) in those experiments where the transparent layer was removed. Albino lemma caryopses also have the ability to translocate assimilates to the endosperm but again little $^{14}$C was recovered in the insoluble material. Evidence for the route taken by pericarp assimilates into the endosperm will be presented in Chapter 4.

Both cv. Midas and Albino lemma caryopses fixed $^{14}$CO$_2$ in the dark although the amount of $^{14}$C recovered from both varieties was considerably less than that recovered after incubation in the light. The dark fixation of $^{14}$CO$_2$ is presumably a result of non-photosynthetic carboxylations catalysed by such enzymes as PEP carboxylase and PEP carboxykinase. PEP carboxykinase was active in the cross cells of barley cv. Julia (Nutbeam 1978). In cv. Midas, the difference in light and dark can be explained by the effect of light on CO$_2$ fixation but this cannot be the
case for Albino lemma which has no chlorophyll in the pericarp. Other factors must affect the amount of CO$_2$ fixed. It may be that PEP carboxylase activity could be related to light or that the higher rates of CO$_2$ fixation in the light in Albino lemma are a result of light-dependent stomatal opening. That is, in the light more CO$_2$ enters the cells where it may be fixed by PEP carboxylase. However, as more $^{14}$C was recovered from Albino lemma caryopses minus the transparent layer after incubation in the light than in the dark this cannot be the full explanation. It seems likely therefore that the carboxylation of CO$_2$ - presumably by PEP carboxylase is light-dependent. The mechanism for this is unknown. In addition, these results confirm the theory that the transparent layer is a barrier to CO$_2$ movement since the amount of $^{14}$C recovered from intact caryopses was half that recovered from caryopses without the transparent layer.

On the basis of O$_2$ exchange measurements, the value of the contribution made by the pericarp to grain filling was estimated at about thirty percent (Nutbeam, 1978). Whilst the experimental conditions used here are not those encountered in vivo, it seems that atmospheric CO$_2$ can be fixed by the cereal pericarp and that the products are translocated to the immature endosperm. Evidence for incorporation of $^{14}$C-labelled photosynthate into starch and cell wall material is especially strong in wheat caryopses minus the transparent layer.
4. LOCATION OF $^{14}$C-LABELLED ASSIMILATES IN CARYOPSSES

4.1 Introduction

In Chapter 3 the fixation of $^{14}$CO$_2$ and subsequent redistribution of $^{14}$C in caryopses of barley and wheat were studied. Some of the $^{14}$C fixed by the pericarp was subsequently recovered from the endosperm but the route by which the $^{14}$C-labelled assimilates reach the endosperm is unknown.

Short distance transport of solutes through tissues involves either the apoplast or the symplast, or both. In apoplastic transport, solutes diffuse through the intercellular spaces and the aqueous phase of cell walls (Briggs and Robertson, 1957). Symplastic transport involves movement of substances between the cytoplasm of cells through plasmodesmata, and may be selective, bidirectional and/or energy-requiring (Spanswick, 1976).

Three possible routes for assimilate entry into the barley endosperm have been postulated (Cochrane and Duffus, 1979). Plasmodesmata have been observed between the cross cells of barley (Cochrane and Duffus, 1979) and wheat (Morrison, 1976) which suggests that assimilates may move via the symplast to the vascular bundle, enter the phloem and then move to the developing endosperm by the route followed by assimilates from other parts of the plant (Fig. 4.1 Route 1). Similar transport mechanisms have been proposed for the transport of assimilates in leaf tissue (Giaquinta, 1983). Studies on wheat leaves indicate that while assimilates travel in the symplast, the final stages involve entry into the apoplast before loading
Fig. 4.1 Possible routes for pericarp assimilate movement to the endosperm. Arrows show the pathway taken by the assimilates, cross hatching shows the sites of CO$_2$ fixation.
into the phloem (Kuo et al., 1974). However, in maize leaves the movement of assimilates between the mesophyll and bundle sheath cells seems to be restricted to a symplastic pathway (Evert et al., 1977, 1978).

Two other possible routes for assimilate entry into the endosperm have been suggested (Cochrane and Duffus, 1979). Metabolites synthesised in the pericarp might reach the endosperm by diffusion through the testa and nucellar epidermis (Fig. 4.1 Route 2). As both these tissues are bounded by cuticles (Morrison, 1975; Cochrane and Duffus, 1979), this mechanism would involve transport across these barriers. Movement of sugars through cuticles has been reported by Gunning and Steer (1975). The second possible route is in the apoplast of the inner part of the transparent layer along broken cell walls and then the apoplast of the crease parenchyma or in the symplast of the chlorophyll-containing cells at either side of the crease (Fig. 4.1 Route 3).

Sugar movement into developing maize kernels has been studied using microautoradiography (Felker and Shannon, 1980). This technique was used here to discover the fate of the products of pericarp photosynthesis in barley caryopses. $^{14}$CO$_2$ was supplied to caryopses, the location of $^{14}$C-labelled assimilates identified and possible routes for assimilate movement in the caryopses described.

4.2 Methods

4.2.1 Plant Material

The barleys cv. Midas and Albino lemma were grown as described
in Section 2.2.1 and caryopses sampled from ears aged as described in sections 2.2 and 2.3.

4.2.2 Incubation of Caryopses with $^{14}$CO$_2$

Four caryopses were incubated with $^{14}$CO$_2$ for 15 min in the light as described in section 3.2.2.

4.2.3 Fixation of Tissues

The $^{14}$C-labelled caryopses were rinsed briefly in distilled water and cut transversely into 1 mm slices with a razor blade. The slices were fixed for 8 - 16 h at room temperature in capped glass vials containing 2.5% (w/v) glutaraldehyde in 0.025 M phosphate buffer, pH 7.2 (25% glutaraldehyde for EM work, EM Scope). Following fixation, the tissue pieces were rinsed twice in 0.025 M phosphate buffer, pH 7.2 for 15 - 20 min and post-fixed in 2% (w/v) OsO$_4$ in 0.025 M phosphate buffer, pH 7.2 for 2 h at room temperature.

4.2.4 Dehydration and Embedding

The tissue slices were rinsed twice in 0.025 M phosphate buffer, pH 7.2 for 15 - 20 min before being dehydrated in a graded ethanol/water series on ice. Ethanol solutions were added consecutively to the tissues for 15 - 20 min and the tissues finally stored in 100% ethanol dried over anhydrous Na$_2$SO$_3$ at 4°C until required.

An acrylic resin, LR White (hard grade, London Resin Company,
P.O. Box 34, Basingstoke) was added to the tissues to give a ratio of 3 parts ethanol to one part resin and the capped vials were left on a rotary mixer (EM Scope) overnight at room temperature. Fresh resin was added the next day to give equal parts of ethanol and resin. At this stage the caps were removed from the vials to allow the ethanol to evaporate. The vial contents were mixed overnight. After two changes of fresh resin, the tissues were placed in gelatin capsules (size 00, EM Scope) in fresh resin and left for 4 h at room temperature before the resin was polymerised at 60°C for 22 h.

4.2.5 Sectioning and Coating of Tissue Sections with Photographic Emulsion

The resin blocks were trimmed and cut with a glass knife on a Reichert Om U3 Ultramicrotome. Nine tissue sections of 2.5 um thickness were placed on drops of water on clean glass microscope slides, the slides dried by heating gently and stored in a dust-free box.

About 20 cm³ of Ilford K5 or L4 photographic emulsion (K5 silver grain size 0.2 μm, L4 grain size 0.15 μm) was heated in a water bath at 40°C in a darkroom illuminated with a Kodak safelight (orange/brown, 6B). An equal volume of warm 2% (v/v) glycerol was added to the emulsion and mixed gently with a glass rod. The diluted emulsion was filtered through muslin into a Coplin jar at room temperature.

The microscope slides were dipped into the emulsion for 2 s, drained, dried vertically for about 20 min at room temperature.
and then dried horizontally. Clean slides and slides with sections of non-radioactive tissues were also coated with emulsion. The slides were kept for 7 - 10 days in light-tight boxes at 4°C.

4.2.6 Development of Photographic Emulsion

The emulsion was developed in 50% (v/v) Phen X developer (Agfa-Gevaert) for 6 - 8 min at room temperature, rinsed in distilled water for 10 s, fixed in 30% (w/v) sodium thiosulphate for 8 min, and washed for 30 min in cold running water. After drying at room temperature, the slides were viewed with a light microscope.

4.2.7 Photography of Tissue Sections

Photographs of the tissues were taken with an Olympus OM1 camera attached to a Vickers Patholux microscope. Kodak Panatomic X (asa 32) was used for black and white photography and Kodak Ektachromes for tungsten light (asa 160) used for colour photography.

4.3 Results

4.3.1 Location of $^{14}$C in Tissues

Caryopses of barleys cv. Midas and Albino lemma were incubated with $^{14}$CO$_2$ in the light, fixed in aqueous solutions, and prepared for microautoradiography. Photographs of the tissues were taken using either black and white film or colour film.
Fig. 4.2 - 4.10 Sections of cv. Midas caryopsis aged 15 days after anthesis. Sections stained with toluidine blue.

Fig. 4.2 Tangential section through the transparent layer.

Fig. 4.3 Tangential section through the transparent layer, cross cells, testa and nucellar epidermis.

Fig. 4.4 Tangential section through the cross cells.

Fig. 4.5 Tangential section through the chlorenchyma and vascular bundle at the crease.

Fig. 4.6 Transverse section through the crease.

Fig. 4.7 Tangential section through the chlorenchyma, testa and nucellar projection.

Fig. 4.8 Tangential section through the nucellar projection.

Fig. 4.9 Tangential section through the crease aleurone.

Fig. 4.10 Transverse section through the starchy endosperm.

Fig. 4.11 Transverse section through a caryopsis of Albino lemma aged 25 days after anthesis.

Fig. 4.12 Transverse section through a caryopsis of cv. Midas incubated with $^{12}\text{CO}_2$.

Key to symbols: a, aleurone; am, amyloplast; ca, crease aleurone; cc, cross cells; ch, chalaza; chl, chlorenchyma; cw, cell walls; e, epidermis; ne, nucellar epidermis; np, nucellar projection; p, phloem; pp, phloem parenchyma; se, starchy endosperm; t, testa; tl, transparent layer; x, xylem.

Arrows indicate silver grains. $^{10\mu\text{m}}$
Silver grains or black dots in the photographic emulsion indicated the presence of $^{14}$C-labelled material in tissues. $^{14}$C-label was found in the epidermis and cell walls of the transparent layer of cv. Midas caryopses aged 15 days after anthesis (Figs. 4.2, 4.3). The walls and small amyloplasts were heavily labelled at this stage of development. Silver grains were also found in the cross cells and in the walls of the testa and nucellar epidermis (Figs. 4.3, 4.4). Chloroplasts in the green wedges of tissue at either side of the crease were also heavily labelled (Fig. 4.5). At the crease, the walls of the testa, nucellar projection and crease aleurone were labelled (Figs. 4.6, 4.7). Figs. 4.8 and 4.9 show tangential sections through the nucellar projection and crease aleurone at higher magnifications. $^{14}$C-label was also present in walls of the phloem and the phloem parenchyma at the crease (Fig. 4.5).

Aleurone cells at the lateral and dorsal sides of the grain did not contain $^{14}$C-label but a few silver grains were found in the cell walls in the middle of the starchy endosperm (Fig. 4.10).

Caryopses of Albino lemma were also exposed to $^{14}$CO$_2$ and processed for microautoradiography. After 15 min exposure to $^{14}$CO$_2$, no silver grains could be found in a mid-caryopsis tissue section (Fig. 4.11) of Albino lemma aged 25 days after anthesis.

Tissue sections of unlabelled cv. Midas and Albino lemma caryopses were also coated with emulsion. A low level of silver grains was observed over the tissue sections and the slides (Fig. 4.12). Slides which were fogged with light did not show
any latent image fading.

The $^{14}$C remaining in caryopses of cv. Midas after fixation and dehydration is shown in Table 4.1. About 26% of the total $^{14}$C per caryopsis was retained in the caryopsis. The remainder was found in the fixation and dehydration solutions.

<table>
<thead>
<tr>
<th>dpm/caryopsis (% of total $^{14}$C in caryopsis)</th>
<th>$^{14}$C extracted during fixation and dehydration</th>
<th>$^{14}$C remaining in caryopsis</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv. Midas caryops</td>
<td>6850 (73.9)</td>
<td>530 (5.8)</td>
<td>1,890 (20.3)</td>
<td>9,270</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1 $^{14}$C extracted from caryopses of cv. Midas during fixation and dehydration plus $^{14}$C remaining in caryopses before embedding in resin. Caryopses, cv. Midas, were sampled from ears cultured in $(U-^{14}$C) sucrose for 12 h in the light, fixed, dehydrated and homogenised in 80% ethanol, washed with water and the insoluble residue heated with 2 M HCl.

4.4 Discussion

If microautoradiography is used to identify the movement of assimilates in the cereal grain, it is essential that the $^{14}$C-labelled material is not extracted or displaced by the experimental procedure. Since the product of pericarp photosynthesis which is transported to the endosperm is most probably sucrose (Nutbeam, 1978) any technique which extracts sucrose during pro-
cessing should ideally not be used. Freeze-substitution with propylene oxide or acetone has been reported to dehydrate and fix plant tissues without displacing alcohol and water-soluble sugars (Fisher and Housley, 1972). About 99% of the 80% ethanol-soluble compounds was retained in leaf petioles after freeze-substitution. Felker and Shannon (1980) also used this technique to study movement of $^{14}$C-labelled assimilates into maize kernels and found that 95% of the total $^{14}$C per kernel piece remained in the tissue after freeze-substitution.

This technique was used here to fix and dehydrate barley caryopses without success. Problems were encountered when the tissues were sectioned. The tissues were not infiltrated fully with the resin and were soft and crumbly. This was probably due either to solvent trapped in the tissue, thus preventing good infiltration by the resin, or inadequate fixation and dehydration. If water is not totally removed from the tissues or from the vials containing the tissue pieces, fixation and dehydration by the solvent may not be adequate.

That photosynthetic assimilates produced in the pericarp can enter the endosperm was shown by quantifying the radioactivity in barley caryopsis tissues after exposure to $^{14}$CO$_2$ in the light (Chapter 3). However, the majority of the $^{14}$C-label recovered was ethanol and water-soluble. Therefore, after fixation of the caryopsis in aqueous solution and dehydration in an ethanol series it is likely that little $^{14}$C will remain in the tissues. About one quarter of the total $^{14}$C per caryopsis remained in the tissues after fixation and dehydration and the
majority of this was in the insoluble fraction. Only $^{14}$C-labelled material categorised as 'insoluble' in the extraction procedure described in Chapter 3 should be unaffected by the fixation and dehydration process unless the fixatives rendered some soluble compounds insoluble. No silver grains were found in tissues of Albino lemma. This was not surprising as very little $^{14}$C was recovered in the insoluble fractions of the tissues (Chapter 3).

In the present work it seems likely that the presence of silver grains is an indication of insoluble rather than soluble material. In the absence of firm information on the distribution of soluble $^{14}$C-labelled compounds in the caryopsis, the pathway involved in the transport of pericarp assimilates cannot be determined conclusively.

The cell walls of many of the tissues of barley caryopses of cv. Midas aged 15 days after anthesis were heavily labelled which suggests that $^{14}$C-labelled photosynthate was used for cell wall synthesis. This was not unexpected as the caryopsis is expanding rapidly at this stage of development (Chapter 2). Whether or not the substrates originally derived from pericarp photosynthesis were derived from the apoplast or symplast is unknown. However, it is possible that some of the $^{14}$C-labelled material in the cell walls may be callose which can be synthesised in response to wounding or glutaraldehyde treatment (Hughes and Gunning, 1980). Glutaraldehyde was used as a fixative in this work. Nevertheless, if callose was synthesised then radioactive substrates must have been available at the site of callose synthesis. Callose has been identified in the thickened cell
walls of the crease aleurone in young barley caryopses using aniline blue induced fluorescence (Cochrane and Duffus, 1980). It has been suggested that callose could act as a temporary storage product which is synthesised if the amount of sugar entering the endosperm exceeds the rate of starch synthesis. Under conditions of high light intensity the products of pericarp photosynthesis, in combination with assimilate entering the grain from the rachis, could saturate the transport system and/or starch synthesis in the endosperm. Thus the conditions prevailing during this set of experiments are likely to have favoured callose synthesis. Although the results suggest that the products of pericarp photosynthesis may be used in cell wall synthesis, the product synthesised remains unknown.

Radioactivity in the cross cells and the chlorophyll containing cells at either side of the crease was mainly in the chloroplasts and was most likely incorporated into starch. $^{14}$C-labelled starch was also observed in small spherical granules in the transparent layer. While it is likely that most of the chloroplast starch is derived from $^{14}$CO$_2$ fixation in the pericarp, some may have come from non-photosynthetic CO$_2$ fixation catalysed by PEP carboxylase (Chapter 6).

The presence of $^{14}$C-label in the walls of the testa and nucellar epidermis suggests that labelled photosynthate may be transported to these tissues and fixed into insoluble material. Little or no $^{14}$C was found inside the cells of these tissues which suggests that the movement of assimilates is not symplastic. Support for this hypothesis comes from ultrastructural studies by Cochrane and Duffus (1979) who were unable to find evidence of plasmo-
desmatal connections between the cross cells and the testa. Since the testa cuticle ends about three cells short of the end of the nucellar epidermis (Cochrane, personal communication) it seems likely therefore that $^{14}$C-labelled compounds could enter the nucellar projection from the testa and/or nucellar epidermis near the chalaza via the gap between the ends of the testa cuticle.

There is also a possibility that $^{14}$C-labelled assimilates could also have reached the nucellar projection via the phloem. Both the phloem and phloem parenchyma walls contained $^{14}$C which could have been derived from the chlorophyll-containing cells at either side of the crease. The transparent layer could also supply assimilates to the phloem. Once in the vascular tissue, $^{14}$C-labelled material could enter the endosperm via the pathway through the chalaza and nucellar projection described by Frazier and Appalanaidu (1965).

As no $^{14}$C was found in aleurone cells at the lateral and dorsal sides of the grain, it seems unlikely that pericarp assimilates move directly through the testa and nucellar epidermis to the endosperm. Of the three possible routes for assimilate movement (Cochrane and Duffus, 1979), it would seem that this route is the shortest and most direct route for entry of pericarp assimilates. However, the results suggest that this is not the case and that the longer route is preferred. That is, assimilates move round the grain in the outer layers to the crease, where they can enter the phloem and/or the nucellar projection. This would be efficient in terms of controlling assimilate supply.
in that the same route would be used by all assimilates enter-
ing the developing grain.
5. SOURCE OF CARBON DIOXIDE FOR PERICARP PHOTOSYNTHESIS

5.1 Introduction

It has been suggested by many workers that the cereal pericarp may function in the refixation of CO₂ respired by the endosperm (Duffus and Rosie, 1973; Radley, 1976; Wirth et al., 1977). This would be advantageous to the grain as, during the grain filling period, respiration by the grain is considerable (Carr and Wardlaw, 1965; Evans and Rawson, 1970; Nutbeam and Duffus, 1978). Fixation of respired CO₂ by pericarp photosynthesis could therefore contribute to the overall carbon economy of the grain.

The suggestion that the pericarp refixes respired CO₂ was derived from the discovery of high levels of phosphoenol pyruvate carboxylase in barley cross cells (Duffus and Rosie, 1973; Nutbeam and Duffus, 1976) and wheat cross cells (Wirth et al., 1977). High levels of PEP carboxylase activity in legume pods have been related to the fixation of CO₂ respired by the seeds (Flinn and Pate, 1970; Hedley et al., 1975; Quebedeaux and Chollet, 1975; Willmer and Johnson, 1976). ¹⁴CO₂ injected into the air space of legume pods and CO₂ respired by the seeds was fixed by the pod wall, particularly the inner epidermis, in the light (Atkins et al., 1977; Flinn et al., 1977). It was concluded that the main function of photosynthesis in legume pods was to refix internally produced CO₂ and thus contribute to the carbon economy of the pod. It was further suggested that the legume pod acts as a temporary storage and assimilatory organ during seed development (Thorne, 1979). The cross cells of the cereal
pericarp may have a similar function.

The source of CO$_2$ for pericarp photosynthesis has not yet been determined. It has been shown that isolated caryopses can fix externally-supplied CO$_2$ in the light (Chapter 3). Whether or not the atmosphere is the sole source of CO$_2$ may be determined by $^{14}$C-labelling the caryopses such that fixation of respired CO$_2$ can be identified. This was investigated in the present work by attempting to follow the fate of $^{14}$CO$_2$ respired by the endosperms and embryos of $^{14}$C-labelled caryopses. In order to label the endosperms, ($^1$U$^{14}$C)sucrose was supplied to detached barley ears in liquid culture. Entry of sucrose into the endosperm, at least in wheat, is considered to be via the phloem of the vascular strand and thence across the chalaza and into the endosperm via the nucellar projection (Frazier and Appalanaidu, 1965). After uptake of labelled sucrose by detached ears, caryopses were detached from these ears and incubated in non-radioactive sucrose in either the light or dark. This system was used in an attempt to discover whether any of the CO$_2$ fixed by the pericarp had originated in the endosperm. For, if pericarp photosynthesis uses CO$_2$ respired by the $^{14}$C-labelled endosperm, the amount of $^{14}$C-label in the caryopsis could be expected to be greater when the caryopsis was exposed to light during the chase period, than when the chase period was dark.

Additionally, $^{14}$C-labelled caryopses (incubated with $^{14}$CO$_2$ as described in Chapter 3) were maintained in either the light or dark for three hours and any redistribution of $^{14}$C-label between
the tissues of the caryopsis noted. Increased amounts of 14C-label would be expected in the pericarp if it refixes respired 14CO2.

5.2 Methods

5.2.1 Plant Material

Ears of the barley cv. Midas or Albino lemma were detached from the parent plants and the flag leaf lamina and sheath removed. The age of the ears was between 20 and 30 days after anthesis and the age of cv. Midas caryopses was determined accurately at the end of the incubation period using the developmental characteristics of the caryopsis described in Table 1. Caryopses of Albino lemma were aged as described in Chapter 2.

5.2.2 Uptake of (U-14C) sucrose by detached barley ears

The stem was cut under water 5 cm below the collar and the ear wiped with 10% (v/v) sodium hypochlorite. Each ear was placed in a sterile glass tube (height 5 cm, diameter 1.1 cm) containing 2 cm3 of sterile 146 mM sucrose and one μCi of (U-14C) sucrose (specific activity 580 mCi mmol-1). A loose cotton wool plug supported the ear and the tubes were placed in an ice-filled box. All incubations were performed at 15°C, relative humidity 60%, in a Sherer Model Cel 255-6 growth cabinet. Lighting was provided by 8 fluorescent tubes supplemented by 2 tungsten lamps. The photon flux density at the level of the ear was 225 μmol.m-2.s-1. Some incubations were carried out in darkness. At the end of the incubation period the ears were either plunged into liquid nitrogen and stored at -20°C or
used as described in 5.2.3 or 5.2.4.

5.2.3 Culture of $^{14}$C-labelled Ears in Unlabelled Sucrose

Following a 12 h dark incubation period in radioactive sucrose, the ears were removed from the tubes, the stems washed in distilled H$_2$O, and placed in tubes containing 2 cm$^3$ of sterile 146 mM-sucrose. Incubation of the ears continued in either the light or dark as described in section 5.2.2. At the end of the incubation period, the ears were plunged into liquid nitrogen and stored at -20°C.

5.2.4 Culture of Caryopses from $^{14}$C-labelled Ears in Unlabelled Sucrose

In order to follow the redistribution of $^{14}$C-labelled material between the endosperm and pericarp, 5 radioactive caryopses were dissected from the middle of 9 ears previously cultured in (U-$^{14}$C) sucrose for 12 h in the dark (section 5.2.2). The age of the caryopses was about 25 days after anthesis. Thirty caryopses were placed upright in sterile glass scintillation vials containing 0.75 cm$^3$ of a sterile solution containing 330 mM sorbitol, 146 mM sucrose, 50 mM Tricine-KOH buffer, pH 7.5. All the vials were plugged with cotton wool and 15 were placed on glass dishes in a Scherer growth cabinet at 15°C and relative humidity 60%. The photon flux density at the level of the caryopses was 225 µmol.m$^{-2}$.s$^{-1}$. Fifteen vials were covered with aluminium foil and placed in a light-tight box in the cabinet. The remaining caryopses were frozen in liquid nitrogen and stored at -20°C.
After 12 h in either the light or dark, the caryopses were frozen in liquid nitrogen and stored at -20°C.

5.2.5 Culture of Isolated Caryopses in (U-\textsuperscript{14}C) Sucrose

Fifteen caryopses of greenhouse-grown barley cv. Midas, aged 25 days after anthesis, were placed in sterile glass scintillation vials containing 0.75 cm\textsuperscript{3} of a sterile solution containing 330 mM sorbitol, 146 mM sucrose, 0.1 \textmu Ci of (U-\textsuperscript{14}C) sucrose (specific activity 580 mCi mmol\textsuperscript{-1}) and 50 mM Tricine-KOH buffer, pH 7.5 in a Scherer growth cabinet for 18 h in the light. The temperature was 15°C and relative humidity 60%. At the end of the incubation period, the caryopses were washed in distilled water, frozen in liquid nitrogen and stored at -20°C.

5.2.6 Extraction of Radioactive Material from \textsuperscript{14}C-labelled Ears

The frozen ears were defrosted at room temperature and 9 ears aged 25 days after anthesis selected. The stem, rachis, and sterile glumes were chopped finely and ground together in 80% ethanol until the tissues had disintegrated. All the grains from the ears, with the exception of 5 grains/ear, were also homogenised in ethanol. The lemma and palea (with awn) were removed from the grain, the transparent layer was dissected from the caryopsis and the remaining tissues, the cross cells, testa, nucellar epidermis and the crease tissue were pooled to give the 'inner layers'. The endosperm and embryo were combined as, at this age, the embryo is small. These tissues were homogenised separately.
Homogenates of stem and whole grains were heated at 70°C and the solid material re-extracted twice with 80% ethanol. The washings and residue were combined, centrifuged at 400 g for 5 min and the supernatants made up to 100 cm$^3$. Distilled water (50 cm$^3$) was added to the pellets, left for 24 h at room temperature and centrifuged at 400 g for 5 min. Both the ethanol- and water-soluble fractions were counted for $^{14}$C-activity and the values pooled to give an estimate of soluble $^{14}$C-labelled material.

After 3 washings with 25 cm$^3$ of distilled water, the insoluble material was digested with 25 cm$^3$ of 2 M HCl at 100°C for about 2 h or until the I$_2$/KI test for starch was negative.

5.2.7 Extraction of Radioactive Material from $^{14}$C-labelled Caryopses

Caryopses cultured in either labelled or unlabelled sucrose solutions were dissected into transparent layers, inner layers, and endosperms plus embryos and extracted as described in section 3.1.3.

5.2.8 Preparation of $^{14}$C-labelled Caryopses for Microautoradiography

Five caryopses from the middle of ears which had been cultured in (U-$^{14}$C) sucrose for 12 h in the light (section 5.2.2) were prepared for microautoradiography as described in Chapter 4.
5.2.9 Chlorophyll Determination

Chlorophyll in green tissues was extracted using the method of Wintermans and De Mots (1965) and the absorbance measured at 654 nm.

5.2.10. Redistribution of $^{14}$C-label in Detached Caryopses

Caryopses were removed from the middle of ears of barley cv. Midas and Albino lemma and 4 caryopses placed in the perspex chamber and incubated with $^{14}$CO$_2$ as described in section 3.2. The age of the caryopses was 25 days after anthesis. The caryopses were then rinsed in distilled water and placed on filter paper moistened with 330 mM sorbitol, 50 mM Tricine KOH, pH 7.5 in a petri dish. The dish was placed in a light-tight box and transferred to a fume cupboard. After 3 h the caryopses were rinsed quickly in distilled water, dissected into transparent layers, inner layers and endosperm plus embryo. The tissues were extracted as described in section 3.3.

The incubation procedure was repeated with four more caryopses but after the incubation with $^{14}$CO$_2$ the caryopses were placed in a fume cupboard for 3 h in the light. Natural daylight was supplemented with a lamp so that the photon flux density was 100 $\mu$mol.m$^{-2}$.s$^{-1}$. After 3 h the caryopses were dissected as described above. Each incubation was repeated at least three times.
5.3 Results

5.3.1 Uptake of (U-\(^{14}\)) sucrose by Detached Barley Ears

Ears of barley cv. Midas were cultured for 6 h in (U-\(^{14}\)) sucrose in either the light or dark. The distribution of \(^{14}\)C-label between tissues of the caryopsis was estimated (Tables 5.1 and 5.2). More \(^{14}\)C-label was found in tissues of caryopses incubated in the dark than in those incubated in the light. In both cases most of the \(^{14}\)C was recovered in the soluble fractions of the tissue extracts. After 6 h no \(^{14}\)C was detected in the insoluble fraction of either the transparent layer or the inner layers. Ear culture in future experiments was therefore extended to 12 h to ensure that all the tissues contained label in both soluble and insoluble material.

5.3.2 Labelling of Tissues from Barley Ears

Initial attempts to \(^{14}\)C-label the endosperms of barley cv. Midas are described in Table 5.3. After culture for 12 h in the dark in (U-\(^{14}\)) sucrose, all the tissues of the ear were \(^{14}\)C-labelled. Most of the label was found in the soluble fractions of the stem (including rachis and sterile glumes) and the grains (53.6% and 43.03% respectively). The soluble fraction contains mainly sugars, amino acids and proteins. Insoluble material includes starch, cell walls and protein. The insoluble fractions of both stem and grains contained \(^{14}\)C-label but only about 3% incorporated was found in these fractions. As these values are from one determination, they can only give an indication of the amounts of \(^{14}\)C-label in each tissue.
<table>
<thead>
<tr>
<th>Tissues</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>420 ± 80</td>
<td>0</td>
<td>420</td>
</tr>
<tr>
<td>Inner layers</td>
<td>440 ± 100</td>
<td>0</td>
<td>440</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>430 ± 40</td>
<td>190 ± 50</td>
<td>620</td>
</tr>
</tbody>
</table>

Table 5.1 ¹⁴C-label in caryopses of cv. Midas ears aged 25 days after anthesis, cultured for 6 h in (U-¹⁴C) sucrose in the light. Values are averages of 3 determinations ± s.d.

Soluble: ethanol-soluble and water-soluble
Insoluble: ethanol- and water-insoluble.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>1,000 ± 100</td>
<td>0</td>
<td>1,000</td>
</tr>
<tr>
<td>Inner layers</td>
<td>920 ± 160</td>
<td>0</td>
<td>920</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>780 ± 260</td>
<td>90 ± 20</td>
<td>1,770</td>
</tr>
</tbody>
</table>

Table 5.2 ¹⁴C-label in caryopses of cv. Midas ears aged 25 days after anthesis, cultured for 6 h in (U-¹⁴C) sucrose in darkness. Values are averaged of 3 determinations ± s.d.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>No.</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layers</td>
<td>15</td>
<td>6,660 (0.87)</td>
<td>240 (0.03)</td>
<td>6,900 (0.9)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>15</td>
<td>11,470 (1.46)</td>
<td>290 (0.04)</td>
<td>11,760 (1.5)</td>
</tr>
<tr>
<td>Endosperms + embryos</td>
<td>15</td>
<td>22,070 (2.8)</td>
<td>4,700 (0.6)</td>
<td>26,850 (3.4)</td>
</tr>
<tr>
<td>Lemmas + paleae</td>
<td>15</td>
<td>41,900 (5.3)</td>
<td>4,110 (0.5)</td>
<td>46,010 (5.8)</td>
</tr>
<tr>
<td>Remaining grains</td>
<td></td>
<td>260,000 (32.6)</td>
<td>14,470 (1.8)</td>
<td>274,470 (34.4)</td>
</tr>
<tr>
<td>Stems, rachis + sterile glumes</td>
<td>3</td>
<td>427,170 (53.6)</td>
<td>3,400 (0.4)</td>
<td>430,570 (56.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>796,560(100)</td>
</tr>
</tbody>
</table>

Table 5.3 $^{14}$C-label in 3 detached ears of barley cv. Midas cultured for 12 h in darkness in (U-$^{14}$C) sucrose (one determination only). Total chlorophyll content of ears was 1,656 µg. Ears were aged 25 days after anthesis.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>No.</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layers</td>
<td>15</td>
<td>25,760</td>
<td>1,850</td>
<td>27,610</td>
</tr>
<tr>
<td>Inner layers</td>
<td>15</td>
<td>39,660</td>
<td>3,400</td>
<td>43,060</td>
</tr>
<tr>
<td>Endosperms + embryos</td>
<td>15</td>
<td>24,300</td>
<td>13,400</td>
<td>32,700</td>
</tr>
<tr>
<td>Lemmas + paleae</td>
<td>15</td>
<td>94,350</td>
<td>5,850</td>
<td>100,200</td>
</tr>
<tr>
<td>Remaining grains</td>
<td>15</td>
<td>686,020</td>
<td>79,010</td>
<td>965,030</td>
</tr>
<tr>
<td>Stems, rachis + sterile glumes</td>
<td>3</td>
<td>122,990</td>
<td>7,770</td>
<td>130,760</td>
</tr>
</tbody>
</table>

**Table 5.4** $^{14}$C-label in 3 detached ears of barley cv. Midas cultured for 12 h in darkness in (U-$^{14}$C) sucrose followed by 12 h in the light in unlabelled sucrose (one determination only). Total chlorophyll content of 3 ears was 2,376 μg. Ears were aged 25 days after anthesis.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>No.</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layers</td>
<td>15</td>
<td>16,450 (1.7)</td>
<td>600 (0.06)</td>
<td>17,050 (1.76)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>15</td>
<td>18,360 (1.9)</td>
<td>1,950 (0.2)</td>
<td>20,310 (2.1)</td>
</tr>
<tr>
<td>Endosperms + embryos</td>
<td>15</td>
<td>49,620 (5.1)</td>
<td>1,950 (0.2)</td>
<td>51,570 (5.3)</td>
</tr>
<tr>
<td>Lemmas + paleae</td>
<td>15</td>
<td>86,520 (8.8)</td>
<td>560 (0.06)</td>
<td>87,080 (8.86)</td>
</tr>
<tr>
<td>Remaining grains</td>
<td></td>
<td>516,190 (52.8)</td>
<td>52,650 (5.4)</td>
<td>568,840 (58.2)</td>
</tr>
<tr>
<td>Stems, rachis + sterile glumes</td>
<td>3</td>
<td>224,890 (23.0)</td>
<td>7,870 (0.78)</td>
<td>232,760 (23.78)</td>
</tr>
</tbody>
</table>

Table 5.5 

14C-label in 3 detached ears of barley cv. Midas cultured for 12 h in darkness in (U-14C) sucrose followed by 12 h in the dark in unlabelled sucrose (one determination only). Total chlorophyll content of 3 ears was 1,860 μg. Ears were aged 25 days after anthesis.
After a further 12 h period in the light in unlabelled sucrose, the distribution of $^{14}$C in the tissues of the ears had altered (Table 5.4). The total $^{14}$C in these ears was greater than in those ears extracted immediately after the pulse of (U-$^{14}$C) sucrose (Table 5.3). However, the amount of chlorophyll in the light-treated ears was greater than in the ears extracted after the $^{14}$C pulse. Although the ears contained varying amounts of radiolabel and were of slightly different ages (varying chlorophyll contents), the percentage of the total $^{14}$C-label in each tissue can be compared.

Considerably less $^{14}$C-label was found in the soluble fraction of the stem (11.1%) after light-treatment (Table 5.4) whilst $^{14}$C in the grains was 88% of the total $^{14}$C present. The majority of the $^{14}$C in the grains was in the soluble fraction (78.7%) and 9.3% in the insoluble fraction.

$^{14}$C-labelled ears which were chased with unlabelled sucrose for 12 h in the dark contained less $^{14}$C than ears maintained in the light for 12 h (Table 5.5). Radioactivity was detected in all tissue fractions and the amount in the grains was about 10% less than in grains from light-treated ears. The stem contained about 24% of the total $^{14}$C which was about twice that found in ears cultured in the light.

Changes in the amount of $^{14}$C-labelled material in the pericarp and endosperm fractions is of interest. Following a chase period of 12 h in the light, 1.2% of the total $^{14}$C was found in the insoluble fraction of the endosperm (Table 5.4) whereas
after dark treatment only 0.2% was recovered in this fraction (Table 5.5). The amount of label in the insoluble fractions of the transparent layer and inner layers was also greater after light-treatment. However, the total $^{14}$C in the endosperm was greater in dark-treated ears than in light-treated ears. The opposite was found to be the case for the pericarp tissues.

Radioactivity in the lemmas and paleae increased from 5.8% of the total $^{14}$C incorporated to 9.03% in light-treated ears (Table 5.4) and 8.86% in dark-treated ears (Table 5.5). Both the soluble and insoluble fractions of these tissues contained $^{14}$C-labelled material but whilst the amount of $^{14}$C in both fractions increased in the light-treated ears, the $^{14}$C in the insoluble fraction of ears chased for 12 h in darkness was almost zero.

The results described in Tables 5.3, 5.4 and 5.5 show that the caryopses, lemmas and paleae, stem, rachis and sterile glumes accumulated $^{14}$C-label. Jenner (1973) noted that these organs, when cultured, could accumulate sucrose to higher levels than those found in vivo. This suggests that $^{14}$C found in the pericarp could have originated in the lemmas, paleae, stem and rachis and could have increased as a consequence of the experimental method. A technique was therefore devised to study caryopses which were detached from the ear. These isolated caryopses would be unaffected by translocation from other organs of the ear. Thus any redistribution of $^{14}$C-label between tissues of the caryopsis should be apparent.
5.3.3 Labelling of Tissues from Detached Caryopses

Initially, caryopses were cultured for 18 h in the light in radioactive sucrose to ensure that dissected caryopses were capable of taking up the culture solution. The rachillas were removed during dissection. The amount of $^{14}$C-label found in each fraction is shown in Table 5.6. Both soluble and insoluble fractions of the tissues contained $^{14}$C-label as did caryopses cultured after removal of the transparent layer. This suggests that the vascular system remained intact following dissection and short-term culture. In subsequent experiments therefore, caryopses were dissected from the ear and then cultured.

<table>
<thead>
<tr>
<th>Intact caryopses</th>
<th>dpm/caryopses</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble</td>
<td>Insoluble</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Transparent layer</td>
<td>940</td>
<td>120</td>
<td>1,060</td>
<td></td>
</tr>
<tr>
<td>Inner layers</td>
<td>640</td>
<td>270</td>
<td>910</td>
<td></td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>660</td>
<td>330</td>
<td>990</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2,960</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.6 $^{14}$C-label in caryopses cv. Midas aged 25 days after anthesis and cultured for 18 h in the light in 0.1 $\mu$Ci of (U-$^{14}$C) sucrose. Values are one determination only. Fifteen caryopses were used.

Ears of cv. Midas were then cultured for 12 h in the dark in radioactive sucrose, caryopses sampled from each ear, and the
amount of $^{14}\text{C}$-label estimated (Table 5.7). $^{14}\text{C}$-labelled material was present in all the tissue fractions but was greatest in the soluble and insoluble fractions of the endosperm (60.4%). There was a higher percentage of counts in the transparent layer than in the inner layers (22.0% and 17.6% respectively) but the difference was not significant.

Caryopses sampled from the ears of cv. Midas cultured for 12 h in darkness in (U-$^{14}\text{C}$) sucrose were subsequently cultured for 12 h in the light, dissected and the tissues extracted. The soluble and insoluble $^{14}\text{C}$-label was estimated in each fraction (Table 5.8) and compared with those in Table 5.7. After light treatment the insoluble fractions of all tissues contained more $^{14}\text{C}$ than the tissues extracted immediately after ear culture. In contrast, the soluble $^{14}\text{C}$-label in tissues was greater in the latter. The $^{14}\text{C}$ totals recovered from caryopses of cultured ears and from caryopses subsequently cultured in the light (Tables 5.7 and 5.8) were not significantly different. Small amounts of radioactivity were also detected in the culture solutions.

Radioactive caryopses sampled from ears cultured for 12 h in darkness were also cultured in unlabelled sucrose for a further 12 h in darkness (Table 5.9). The total $^{14}\text{C}$ in the caryopses was about 9% less than in caryopses extracted immediately after 12 h in (U-$^{14}\text{C}$) sucrose, and was also less than caryopses cultured for 12 h in the light. A greater percentage of the radioactivity was found in the soluble fractions than in insoluble material. In comparison with caryopses cultured in the light
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble (dpm)</th>
<th>Insoluble (dpm)</th>
<th>Total (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>2,650 ± 180</td>
<td>90 ± 15</td>
<td>2,740 (22.0)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>2,020 ± 400</td>
<td>170 ± 10</td>
<td>2,190 (17.6)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>4,060 ± 200</td>
<td>3,470 ± 140</td>
<td>7,530 (60.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12,460 (100)</td>
</tr>
</tbody>
</table>

Table 5.7 14C-label in caryopses cv. Midas aged 25 days after anthesis after detached ear culture for 12 h in darkness in (U-14C) sucrose. Values are averages of 3 determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble (dpm)</th>
<th>Insoluble (dpm)</th>
<th>Total (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>1,870 ± 180</td>
<td>270 ± 30</td>
<td>2,140 (16.6)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>1,520 ± 250</td>
<td>790 ± 180</td>
<td>2,310 (17.9)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>3,130 ± 500</td>
<td>5,310 ± 180</td>
<td>8,440 (65.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12,890 (100)</td>
</tr>
</tbody>
</table>

Table 5.8 14C-label in caryopses cv. Midas aged 25 days after anthesis after culture for 12 h in the light of caryopses sampled from detached ears previously cultured in (U-14C) sucrose for 12 h in the dark. Values are averages of 3 determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble (dpm)</th>
<th>Insoluble (dpm)</th>
<th>Total (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>3,050 ± 630</td>
<td>120 ± 30</td>
<td>3,170 (28.5)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>2,230 ± 870</td>
<td>160 ± 35</td>
<td>2,390 (21.4)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>3,100 ± 470</td>
<td>2,480 ± 1,200</td>
<td>5,580 (50.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11,140 (100)</td>
</tr>
</tbody>
</table>

Table 5.9 14C-label in caryopses cv. Midas aged 25 days after anthesis after culture for 12 h in darkness of caryopses sampled from detached ears previously cultured in (U-14C) sucrose for 12 h in darkness. Values are averages of 3 determinations ± s.d.
# Table 5.10 14C-label in caryopses of cv. Albino lemmata aged 25 days after anthesis after detached ear culture for 12 h in darkness in (U-14C) sucrose.

Values are averages of 3 determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>860 ± 240 (17.0)</td>
<td>60 ± 30 (1.2)</td>
<td>920 (18.2)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>740 ± 210 (14.6)</td>
<td>30 ± 10 (0.6)</td>
<td>770 (15.2)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>2,250 ± 810 (44.3)</td>
<td>1,130 ± 100 (22.3)</td>
<td>3,380 (66.6)</td>
</tr>
</tbody>
</table>

Table 5.11 14C in caryopses of cv. Albino lemmata aged 25 days after anthesis following culture for 12 h in the light of caryopses sampled from detached ears previously cultured in (U14-C) sucrose for 12 h in darkness. Values are averages of 3 determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>1,120 ± 40 (20.8)</td>
<td>210 ± 20 (3.9)</td>
<td>1,330 (24.7)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>760 ± 150 (14.1)</td>
<td>260 ± 100 (4.8)</td>
<td>1,020 (18.9)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>2,160 ± 440 (40.2)</td>
<td>870 ± 165 (16.2)</td>
<td>3,030 (56.4)</td>
</tr>
</tbody>
</table>

Table 5.12 14C-label in caryopses of cv. Albino lemmata aged 25 days after anthesis following culture for 12 h in darkness of caryopses sampled from detached ears previously cultured in U14-C sucrose for 12 h in darkness. Values are averages of 3 determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>760 ± 210 (14.6)</td>
<td>40 ± 10 (0.8)</td>
<td>740 (15.4)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>620 ± 190 (13.0)</td>
<td>70 ± 20 (1.5)</td>
<td>690 (14.5)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>2,390 ± 550 (50.0)</td>
<td>960 ± 380 (20.1)</td>
<td>3,350 (70.1)</td>
</tr>
</tbody>
</table>

Table 5.13 14C-label in caryopses of cv. Albino lemmata aged 25 days after anthesis following culture for 12 h in darkness of caryopses sampled from detached ears previously cultured in U14-C sucrose for 12 h in darkness. Values are averages of 3 determinations ± s.d.
it was evident that a greater proportion of the $^{14}\text{C}$ was in the soluble fractions of dark-cultured caryopses than in light-cultured caryopses. However, after light-treatment about 40% of the total $^{14}\text{C}$ was in the insoluble fraction of the endosperm whereas after dark-treatment this value was halved. This value was also less than in caryopses extracted immediately after ear culture.

Culture of caryopses from $^{14}\text{C}$-labelled ears was repeated for the barley Albino lemma (Table 5.10). Although the total $^{14}\text{C}$ recovered was less than half the total $^{14}\text{C}$ in cv. Midas caryopses, the distribution of $^{14}\text{C}$ between tissues was similar for the two barleys. However, the insoluble fraction of the endosperm of Albino lemma contained less $^{14}\text{C}$ than the same fraction of cv. Midas.

Comparison of light- and dark-treated caryopses of Albino lemma showed that similar amounts of $^{14}\text{C}$ remained in the caryopses after culture (Tables 5.11 and 5.12). Slightly more $^{14}\text{C}$ was found in the insoluble fraction of the endosperm of light-treated caryopses than in that of dark-treated caryopses but the difference was not significant. Similarly, the $^{14}\text{C}$ levels in the inner layers and transparent layer after light-treatment were not significantly different from those after dark-treatment.

5.3.4 Location of $^{14}\text{C}$ in Caryopsis Tissues

Caryopses of cv. Midas aged 25 days after anthesis were sampled from ears cultured for 12 h in (U-$^{14}\text{C}$) sucrose in the light, fixed, sectioned and the location of insoluble $^{14}\text{C}$-labelled
compounds detected using microautoradiography. Ethanol- and water-soluble compounds were extracted during fixation.

Silver grains were found in the starchy endosperm and were concentrated over starch granules (Fig. 5.1 and 5.2). The cell walls of the starchy endosperm were also labelled. Little $^{14}$C was found in the outer layers of the caryopsis with the exception of the cross cells which had silver grains over the chloroplasts (Fig. 5.3).

5.3.5 $^{14}$CO$_2$ re-fixation by Detached Caryopses

The amount of $^{14}$C-label in tissues of cv. Midas caryopses was estimated after an initial pulse of $^{14}$CO$_2$ followed by a 3 h chase period in $^{12}$CO$_2$ in either the light or dark. It has been shown that the total $^{14}$C per caryopsis immediately after the pulse of $^{14}$CO$_2$ was 102 120 dpm (Table 5.13).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>dpm/caryopsis ( % of total $^{14}$C recovered)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>5540 ± 2660 (6.4)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>80370 ± 3730 (78.7)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>12660 ± 950 (12.4)</td>
</tr>
</tbody>
</table>

Table 5.13 $^{14}$C in caryopses of cv. Midas aged 25 days after anthesis after 15 min in the light with $^{14}$CO$_2$. Values are averages of 3 separate determinations ± s.d. (These results are from Table 3.1).
Fig. 5.1 Transverse section of endosperm of cv. Midas.

Fig. 5.2 Transverse section of starchy endosperm cells of cv. Midas.

Fig. 5.3 Transverse section of outer tissues of cv. Midas caryopsis.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>dpm/tissue (％ of total (^{14}\text{C}) recovered/caryopsis)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>4,990 ± 790 (36.3)</td>
<td>250 ± 50 (1.8)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>3,360 ± 1,030 (24.5)</td>
<td>1,480 ± 560 (10.8)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>3,210 ± 920 (23.4)</td>
<td>440 ± 140 (3.2)</td>
</tr>
</tbody>
</table>

Table 5.14 \(^{14}\text{C}\)-label in cv. Midas caryopses after 15 min in the light with \(^{14}\text{CO}_2\) followed by 3 h in the light in \(^{12}\text{CO}_2\). Values are averages of 3 separate determinations ± s.d. The caryopses were aged 25 days after anthesis.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>dpm/tissue (％ of total (^{14}\text{C}) recovered/caryopsis)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Transparent layer</td>
<td>1,600 ± 400 (34.0)</td>
<td>170 ± 20 (3.6)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>1,130 ± 290 (24.0)</td>
<td>170 ± 90 (3.6)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>1,510 ± 590 (32.0)</td>
<td>130 ± 30 (2.8)</td>
</tr>
</tbody>
</table>

Table 5.15 \(^{14}\text{C}\)-label in cv. Midas caryopses after 15 min in the light with \(^{14}\text{CO}_2\) followed by 3 h in darkness with \(^{12}\text{CO}_2\). Values are averages of 3 separate determinations ± s.d. The caryopses were aged 25 days after anthesis.
Table 5.16 14C in caryopses of Albino lemma aged 25 days after anthesis after 15 min in the light with 14CO2 followed by 3 h in the light with 12CO2. Values are averages of 3 separate determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>5.140 ± 1.670 (47.7)</td>
<td>140 ± 50 (1.3)</td>
<td>5,280 (49.0)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>1.990 ± 290 (18.5)</td>
<td>150 ± 40 (1.4)</td>
<td>2,140 (19.9)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>3,360 ± 690 (30.6)</td>
<td>60 ± 10 (0.5)</td>
<td>3,360 (31.1)</td>
</tr>
</tbody>
</table>

10,780(100)

Table 5.17 14C in caryopses of Albino lemma aged 25 days after anthesis after 15 min in the light with 14CO2 followed by 3 h in darkness with 12CO2. Values are averages of 3 separate determinations ± s.d.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparent layer</td>
<td>3,840 ± 620 (37.5)</td>
<td>520 ± 100 (5.1)</td>
<td>4,360 (42.6)</td>
</tr>
<tr>
<td>Inner layers</td>
<td>2,650 ± 610 (25.9)</td>
<td>210 ± 110 (2.0)</td>
<td>2,860 (27.9)</td>
</tr>
<tr>
<td>Endosperm + embryo</td>
<td>2,690 ± 680 (26.3)</td>
<td>330 ± 170 (3.2)</td>
<td>3,020 (29.5)</td>
</tr>
</tbody>
</table>

10,240(100)
After 3 h in the light in $^{12}$CO$_2$, the total $^{14}$C per caryopsis had dropped to 13730 dpm which is about 13% of the original radioactivity (Table 5.14). $^{14}$C was lost from all tissues but the majority was lost from the inner layers, in particular the soluble fraction. The soluble fraction of the endosperm also contained considerably less $^{14}$C after the chase period. Both the transparent layer and endosperm had more $^{14}$C in their insoluble fractions but the same fraction of the inner layers contained about half the $^{14}$C present immediately after the $^{14}$C pulse.

After 3 h in the dark, the total $^{14}$C per caryopsis was 4710 dpm, about one third of the total $^{14}$C recovered per caryopsis after 3 h in the light (Table 5.15). All the tissue fractions contained significantly less $^{14}$C after 3 h in the dark but the percentage of the total $^{14}$C in the soluble fraction of the endosperm had increased to 32.0% of the total radioactivity. The most notable loss of $^{14}$C was from the insoluble fraction of the inner layers. The distribution of the radioactivity between the endosperm and its surrounding layers (transparent layer and inner layers) altered during the chase periods so that a greater proportion of the radioactivity was found in the endosperm after 3 h in the dark than after 3 h in the light.

$^{14}$C in caryopses of Albino lemma was also estimated after exposure of the caryopses to a pulse of $^{14}$CO$_2$ followed by chase periods in $^{12}$CO$_2$ in either the light or dark. The total $^{14}$C recovered per caryopsis was similar for both treatments (Tables 5.16 and 5.17) and the distribution of $^{14}$C between the tissues was not significantly different. However, the amounts of $^{14}$C
in the soluble and insoluble fractions of each tissue were different for the two treatments. More $^{14}$C was recovered from the insoluble fractions of the tissues after dark treatment than after 3 h in the light.

As with cv. Midas, the transparent layer of **Albino lemma** contained more $^{14}$C-label than the other tissues after 3 h in either the light or dark. The soluble fractions of the transparent layers of both barley varieties had more $^{14}$C after 3 h in the light than any other tissue fraction. In contrast to cv. Midas, more $^{14}$C was recovered from the inner layers of **Albino lemma** after 3 h in darkness than after 3 h in the light.

5.6 Discussion

Culture of cereal ears in radioactive sucrose solutions was first described by Buttrose and May (1959) and Jenner (1968) who found that $^{14}$C-sucrose entered the grains and supported starch synthesis in the endosperm. Using a similar technique the uptake of (U-$^{14}$C) sucrose by detached ears of the barleys cv. Midas and **Albino lemma** has been reported and the subsequent redistribution of $^{14}$C-label in caryopses determined.

The culture medium contained 50 mg cm$^{-3}$ sucrose (0.146 M) which was used by Jenner (1968) to maintain detached wheat ears in darkness for 48 h. Raising the external sucrose concentration above a maximum value (40 gm cm$^{-3}$ for wheat) did not alter the influx of sucrose into the endosperm (Jenner, 1970). However, the sucrose concentrations in other tissues of the ear were related to the external sucrose concentration. Jenner and Rathjen
(1972 a, b) concluded that the entry of sucrose into the grain is limited either by the grain itself or by the passage of sucrose into the grain. Presumably a similar limitation on sucrose influx exists in barley which suggests that sucrose entry and distribution within the caryopsis is independent of external sucrose concentrations above a certain level.

Long-term culture of detached ears has been described by Donovan and Lee (1977 and 1978) using a medium containing sugars, a nitrogen source, and essential minerals and growth factors. It has been shown by these authors and Jenner and Rathjen (1978), Campbell et al. (1981), and Barlow et al. (1983) that detached ears grown in liquid culture are comparable with those on intact plants and that the transport system in the ear is unaffected.

Culture of detached ears was used to study the possible refixation of CO₂ respired by the endosperm by pericarp photosynthesis. The aim was to label the endosperm with ¹⁴C and follow the redistribution of ¹⁴C in the caryopsis in the light. Refixation of respired ¹⁴CO₂ should thus give increased ¹⁴C levels in the pericarp. However, it was apparent from the initial experiments with ears of cv. Midas cultured for 6 h in the light that not only was the starch in the endosperm unlabelled but the pericarp tissues contained considerable amounts of ¹⁴C. These findings agree with Buttrose and May (1959) who showed that, after 5 h in ¹⁴C-sucrose, little ¹⁴C was detected in insoluble material in barley caryopsis. Jenner (1973) also found ¹⁴C in the transparent layer of wheat and in caryopses minus the transparent layer after 8 h culture in ¹⁴C-sucrose.
The culture of barley ears in the experiments described here was therefore extended to 12 h and the distribution of $^{14}$C in caryopses immediately after the $^{14}$C pulse was compared with that in caryopses removed from ears which had had an additional 12 h in unlabelled sucrose in either the light or dark.

The results with detached ears of cv. Midas incubated with $^{14}$C-sucrose showed that more $^{14}$C was recovered in the pericarps of ears cultured in the light than in those maintained for 24 h in darkness. However, the increased $^{14}$C could have been due to translocation from the glumes, stem and rachis. The radioactivity in these tissues was especially depleted after culture for 12 h in the light. Labelled material in the stem moved to the grains as the culture time increased. Although these experiments did suggest that the pericarp might be involved in refixing CO$_2$, the presence of $^{14}$C-label in tissues of the ear other than the caryopsis makes the results difficult to interpret.

One conclusion that can be made is that light stimulates the incorporation of $^{14}$C into insoluble material. The majority of the insoluble material in the endosperm and inner layers is probably starch as, at 25 days after anthesis, grain filling is rapid (Chapter 2). Microautoradiographic studies on the location of $^{14}$C-label in the endosperm indicated that the starch granules were highly labelled. Only a small amount of label was identified in the cell walls. It seems likely that the majority of the $^{14}$C in the endosperm is incorporated into starch although it is possible that some is incorporated into protein.
In order to study the redistribution of $^{14}$C between the endosperm and pericarp, caryopses were detached from $^{14}$C-labelled ears and cultured in unlabelled sucrose in either the light or dark. The method of culture was adopted from the technique described by Gifford and Bremner (1981a).

They used half caryopses of wheat or caryopses minus their transparent layers as $^{14}$C was not found in the caryopses if the transparent layer was intact. Here, the caryopses were dissected from the ears under water to prevent air entering the vascular system and intact caryopses had the ability to take up $^{14}$C-sucrose from the culture solution.

The results of caryopsis culture of cv. Midas showed that although more $^{14}$C remained in the caryopsis after light-culture than after dark-culture, the pericarp did not contain significantly more $^{14}$C after light-culture. This suggests that this tissue did not refix internal $^{14}$CO$_2$ respired by the endosperm. However the experimental procedure was further modified by subjecting short-term pre-labelled (with $^{14}$CO$_2$) detached caryopses to longer-term chases in $^{12}$CO$_2$ in either the light or dark. By this method it was hoped to follow any refixation by pericarp photosynthesis of $^{14}$CO$_2$ respired by the endosperm. The results showed that more $^{14}$C was recovered from caryopses chased with $^{12}$CO$_2$ in the light and that the pericarp tissues contained significantly more $^{14}$C than caryopses chased in the dark. There is therefore some evidence to suggest that the cross cells can fix internally produced CO$_2$ and reduce respiratory losses of carbon in the developing grain.
It seems likely that the major source of $\text{CO}_2$ for pericarp photosynthesis is the atmosphere (Chapter 3). Whether or not the husk of the cereal grain has a role in the fixation of $\text{CO}_2$ respired by the caryopsis was not investigated here. Certainly it has been shown that stomata are present on the lemma and palea of barley (Miskin and Rasmusson, 1970) and the glumes of wheat can fix atmospheric $\text{CO}_2$ (Bremner and Rawson, 1972).

However, the results from detached caryopsis culture do suggest that there is light stimulation of starch synthesis in all the tissues sampled. This is particularly noteworthy in the endosperm and confirms the results of Gifford and Bremner (1981 b) who noted light stimulation of starch synthesis in wheat caryopses. The effect could be blocked by the addition of inhibitors of photosynthesis or by removal of the cross cells. Further work suggested that oxygen stimulated the incorporation of sucrose into starch. The experiments with Albino lemma did not show any effect of light on starch synthesis since significant differences between light- and dark-cultured caryopses were not observed. As Albino lemma caryopses have no chlorophyll in their cross cells and therefore no photosynthesis, these findings support the hypothesis that oxygen stimulates starch synthesis.

It has been suggested that oxygen may be involved in the regulation of sink activity in the developing grain via its role in oxidative phosphorylation (Duffus and Cochrane, 1982). The source of oxygen could be photosynthesis in the cross cells (Nutbeam and Duffus, 1978, Gifford and Bremner, 1981 b), photosynthesis by other parts of the plant whereby the oxygen enters
the endosperm in solution via the phloem stream, and/or the atmosphere via stomata of the pericarp epidermis or other parts of the plant. In immature detached barley caryopses 25 days after anthesis, the amount of oxygen evolved in cross cell photosynthesis was sufficient to supply that taken up in respiration (Nutbeam and Duffus, 1978). In the caryopsis of Albino lemma there was always net oxygen uptake in the light. It must be concluded that, at least in this barley, any oxygen required for sink activity is derived from sources other than cross cell photosynthesis. It may be that the mutant has some special adaptations which compensate for the lack of chlorophyll in its cross cells. That cross cells are not the sole source of oxygen for grain filling is indicated by the observation that pericarp photosynthesis in most cultivars continues for not more than the first two-thirds of the maturation period although grain weight continues to increase beyond this period (Duffus and Rosie, 1973).

Since oxygen is thought to be sparingly soluble in cuticular material and the epidermal stomata are few in number, it seems likely that most of the oxygen produced by cross cell photosynthesis remains within the caryopsis. It has been suggested that the oxygen diffuses round the caryopsis in the gap between the transparent layer and the cross cells, and enters the endosperm via the chazalal region and the nucellar projection at the crease (Cochrane and Duffus, 1979). It may be therefore that the pericarp regulates endosperm activity by controlling the oxygen supply.
There is also some evidence from the results of the $^{14}\text{CO}_2/^{12}\text{CO}_2$ pulse chase of isolated caryopses which suggests that starch in the cross cells of cv. Midas may be turned over. It seems likely that pericarp photosynthetic assimilates are the main substrate for grain respiration since by far the greatest proportion of $^{14}$C-label was lost from the cross cell soluble fraction. Label was also lost from the insoluble fraction of the cross cells, which suggests that starch in these cells can be turned over whilst that in the transparent layer and endosperm was not degraded. MacGregor et al. (1972) also noted the disappearance of pericarp starch.

Further evidence in support of starch turnover comes from the detached ear experiments in which it was shown that incorporation into the endosperm insoluble fraction was greater 12 h after the start of the experiment than after 24 h in darkness. This suggests that there may be some degradation of endosperm starch during grain development under certain conditions.

In conclusion it appears that although the developing caryopsis can fix atmospheric CO$_2$, the evidence for refixation of respired CO$_2$ by pericarp photosynthesis is not conclusive. It may be that a major role of pericarp photosynthesis is to control the oxygen supply to the endosperm. Oxygen evolution by the cross cells is probably responsible for the stimulation of endosperm starch synthesis observed in the light. Thus the most important function of the pericarp may be the supply of oxygen for starch synthesis and not the traditionally ascribed role of conserving respiratory CO$_2$. 
6.1 Introduction

Little is known about the biochemical functions of the trans­parent layer or outer pericarp of cereals. Whilst changes in the pericarp have been described using histological techniques (Percival, 1921; Krauss, 1933), there have been few biochemical studies of this tissue.

Starch granules are present in the transparent layer of cereal pericarps during the first few days of grain-filling but by about 14 days after anthesis have disappeared (MacGregor et al., 1972; Morrison, 1976; MacGregor et al. (1972) suggested that the starch granules act as a temporary energy store for the developing endosperm and are degraded by α-amylase which is active in immature barley pericarps. It seems therefore that the transparent layer has starch synthesising ability during early development but this ability disappears as the caryopsis matures.

The cross cells of immature cereal pericarps also contain starch in their chloroplasts during early grain development (May and Buttrose, 1959; MacGregor et al., 1972). The source of assimilates for pericarp starch synthesis could be cross cell photosynthesis. Both the C_3 and C_4 pathways of photosynthesis have been shown to operate in the cross cells (Nutbeam and Duffus, 1976; Wirth et al., 1977), and enzymes of C_3 and C_4 photosynthesis have been identified in this tissue.

In storage tissues where sucrose is thought to be the major
substrate for starch synthesis, it is considered that sucrose synthase plays an important role in the breakdown of sucrose (Akazawa, 1972). Sucrose synthase activity in developing maize endosperm (Tsai et al., 1970; Hawker, 1971), barley (Baxter and Duffus, 1973) and rice (Perez et al., 1975) was appreciably higher than soluble invertase activity throughout grain development. However, high invertase activity was observed in the pedicel and placento-chalazal tissues of maize (Shannon and Dougherty, 1972) and bound invertase in rice grains was thought to be responsible for the hydrolysis of sucrose during entry into the endosperm (Perez et al., 1975). In contrast, Jenner (1974) and Sakri and Shannon (1975) showed that in wheat, sucrose was not hydrolysed as it entered the endosperm.

An attempt has therefore been made in the present work to describe some of the key biochemical functions of immature pericarp tissues. Thus the activities of both sucrose synthase (E.C.2.4.1.13) and invertase (E.C.3.2.1.26) were measured in the transparent layer and cross cells/testa of rapidly growing caryopses.

In addition, the activities of the carboxylating enzymes, RUBP carboxylase and PEP carboxylase, were studied in order to investigate further the process of CO₂ fixation in the transparent layer and cross cells of the immature pericarp.
6.2 Methods

6.2.1 Extraction of sucrose synthase and invertase activity

Twenty to 25 grains were removed from the middle of 4 ears of the barleys cv. Midas or Albino lemma and either the transparent layer or the inner layers dissected from the caryopses. Grains aged about 25 days after anthesis were used.

The tissues were placed immediately in 1 cm³ of ice-cold isolation medium (50 mM 2-(N-morpholino) ethane sulphonic acid (MOPS), pH 7.0) and homogenised in a hand-held all-glass tissue grinder. The supernatant after settling was removed and the insoluble material re-extracted in 1 cm³ of isolation medium. Both extracts and remaining insoluble material were combined and centrifuged in a swing-out rotor at 10000 g for 15 min at 4°C. The supernatant was used as the enzyme extract.

6.2.2 Assay of sucrose synthase

The method used was that of Avigad and Milner (1966) adapted for cereal caryopses by H.C. Riffkin (personal communication). Enzyme activity was assayed in the direction of sucrose breakdown and the initial reactants were sucrose and uridine diphosphate (UDP). The release of fructose from sucrose was followed colorimetrically using the Somogyi-Nelson assay for reducing sugars (Nelson, 1944).

The reaction was initiated by the addition of 0.1 cm³ of enzyme extract to an incubation tube containing 0.5 cm³ of 0.1M MOPS, pH 7.0 and 0.5 M sucrose, 0.1 cm³ of UDP in 0.1 M MOPS, pH 7.0,
of 0.1 M NaF in 0.1 M MOPS, pH 7.0 and 0.25 cm$^3$ of distilled water. A control tube contained enzyme extract and UDP but no added sucrose. The tubes were incubated at 25°C and at 0, 5, 10, 15 and 25 min 0.15 cm$^3$ was withdrawn and mixed with 0.85 cm$^3$ of fresh 1mM N-ethyl maleimide to stop the reaction. After heating for 1 min at 100°C, 1 cm$^3$ of Somogyi-Nelson copper reagent was added. The tubes were mixed well, boiled for 10 min and 1 cm$^3$ of Somogyi-Nelson arsénomolybdate reagent added to each tube. The volume was made up to 10 cm$^3$ with distilled water and 15 min later the absorbance at 500 nm measured. A graph of known concentrations of an equimolar glucose/fructose mixture versus absorbance at 500 nm was prepared with each enzyme assay and the amount of reducing sugars in each sample calculated. The assay was replicated three times for each tissue.

6.2.3 Assay of soluble invertases

The activity at pH 7.0 of soluble invertase in the pericarp tissues was measured in conjunction with sucrose synthase. An incubation tube containing 0.5 cm$^3$ of 0.5 M sucrose in 0.1 M MOPS, pH 7.0, 0.05 cm$^3$ of 0.1 M NaF and 0.35 cm$^3$ of distilled water was incubated at 25°C and the reaction initiated with 0.1 cm$^3$ of enzyme extract (section 6.2.1). A control tube contained enzyme but no added sucrose. At 0, 5, 10, 15 and 25 min intervals 0.15 cm$^3$ was withdrawn and mixed with 0.85 cm$^3$ of 1 mM N-ethyl maleimide. The amount of reducing sugars in each tube was estimated as described above.
Soluble invertase activity was also measured at pH 5.0. The method was a modification of that described above for neutral invertase except that 0.1 M acetate buffer, pH 5.0 was used in place of 0.1 M MOPS, pH 7.0. The results were corrected for activity measured in the absence of added sucrose. The assay was replicated three times for each tissue.

6.2.4 Extraction of RuBP carboxylase and PEP carboxylase

Between 20 to 30 grains were removed from the middle of 4 ears of cv. Midas or Albino lemma and either the transparent layer or inner layers dissected from the caryopses. Grains aged about 25 days after anthesis were used.

The tissues were homogenised in a hand-held all-glass tissue grinder in 2 cm³ of ice-cold isolation medium (1 mM MnCl₂, 1 mM MgCl₂, 10 mM mercaptoethanol, 330 mM sorbitol, 50 mM Tricine, pH 7.5). The extract was centrifuged at 400 g for 2 min and the pellet re-extracted with 1 cm³ of the isolation medium. The extracts and pellet were recombined and centrifuged as before and the supernatant was used as the enzyme extract.

6.2.5 Assay of RuBP Carboxylase

A modified form of the assay of Bahr and Jensen (1978) was used. A tube containing 1 cm³ of incubation medium (25 mM MgCl₂, 10 mM mercaptoethanol, 25 mM Hepes, pH 7.8, 19.8 mM NaHCO₃, 14 µCi of NaH¹⁴CO₃ (specific activity 58 mCi mmol⁻¹) and 0.1 cm³ of enzyme extract was placed in a water bath at 25°C for 4 min. The photon flux density was 700 µmol m⁻² s⁻¹.
To initiate the carboxylase reaction, 0.1 cm\(^3\) of 3.6 mM ribose-5-phosphate (R5P) and 3.6 mM adenosine triphosphate (ATP) in the incubation buffer was added. Immediately after mixing and at 5 and 10 min intervals 0.1 cm\(^3\) was withdrawn from the tube and mixed with 0.5 cm\(^3\) of 5% (w/v) trichloroacetic acid (TCA) in ethanol. Acid-stable compounds were counted for \(^{14}\)C-label.

The ethanolic mixtures were heated at 70°C for 15 min to drive off unfixed \(^{14}\)CO\(_2\). Samples taken before and after heating at 70°C were counted to ensure that all unfixed \(^{14}\)CO\(_2\) was driven off. After 15 min the \(^{14}\)C-level in each tube remained constant. A 0.1 cm\(^3\) sample was added to 15 cm\(^3\) of Triton X-100/toluene liquid scintillation fluid and the vials counted for 10 min in a Beckman LS100C liquid scintillation spectrometer. The efficiency of counting was about 80 percent.

Values obtained for controls containing either substrate or enzyme were subtracted from those obtained with both enzyme and substrate present. The assays were replicated three times. The linearity of the reaction rate was checked by sampling at 1 min intervals for 10 min.

6.2.6 Assay of PEP carboxylase

The modification of the method of Slack and Hatch (1967) described by Nutbeam (1978) was used to assay PEP carboxylase in pericarp tissues. Preparation of the enzyme extract was as described in Section 6.2.4. After mixing 0.1 cm\(^3\) of enzyme extract with 1 cm\(^3\) of the incubation medium (Section 6.2.5),
0.1 cm$^3$ of 80 mM sodium glutamate in incubation buffer, pH 7.8 was added. The tubes were placed in a water bath at 25°C for 5 min to equilibrate and the reaction initiated by the addition of 0.1 cm$^3$ of 33 mM PEP in incubation buffer. The photon flux density was 700 μmol. m$^{-2}$.s$^{-1}$.

Immediately after mixing and at 5 and 10 min intervals, 0.1 cm$^3$ samples were mixed with 0.5 cm$^3$ of 5% TCA in ethanol, heated at 70°C for 15 min and the acid-stable $^{14}$C-labelled compounds counted for $^{14}$C activity (Section 6.2.5). The linearity of the reaction rate was checked by sampling at 1 min intervals for 10 min.

6.2.7 Chlorophyll determinations

The total chlorophyll content of the extract containing the cross cells was estimated using the method of Arnon (1949).

6.3 Results

6.3.1 Sucrose synthase and soluble invertases

The inner layers of both barley cultivars, cv. Midas and Albino lemma had sucrose synthase activity (Tables 6.1 and 6.2). Those tissue fractions were composed of cross cells, testa and nucellar epidermis and the crease region. Any enzyme activity detected in this fraction cannot therefore be attributed to one particular tissue. Sucrose synthase activity in the inner layers of Albino lemma caryopses was greater and more variable than that detected in cv. Midas. No sucrose synthase activity was found in the transparent layer of cv. Midas and only low
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Transparent layer</th>
<th>Inner layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose synthase</td>
<td>0</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>Soluble invertase pH 5.0</td>
<td>0.125 ± 0.03</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>Soluble invertase pH 7.0</td>
<td>0</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

Table 6.1 Enzyme activity in tissues of barley cv. Midas caryopses aged 25 days after anthesis. Each value is the mean of 3 replicates ± s.d.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Transparent layer</th>
<th>Inner layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose synthase</td>
<td>0.03 ± 0.02</td>
<td>0.35 ± 0.19</td>
</tr>
<tr>
<td>Soluble invertase pH 5.0</td>
<td>0.02 ± 0.01</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Soluble invertase pH 7.0</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>

Table 6.2 Enzyme activity in tissues of barley Albino lemma caryopses aged 25 days after anthesis. Each value is the mean of 3 replicates ± s.d.

A second control tube contained enzyme extract and sucrose but no added UDP (a measure of invertase activity). The values obtained were subtracted from the value for the tube containing both sucrose and UDP to give an estimate of sucrose synthase.
<table>
<thead>
<tr>
<th></th>
<th>nmol CO₂ min⁻¹ tissue⁻¹</th>
<th></th>
<th>nmol CO₂ min⁻¹ mg chlorophyll⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transparent layer</td>
<td>Inner layers</td>
<td>Inner layers</td>
</tr>
<tr>
<td>RuBP carboxylase</td>
<td>0</td>
<td>0.1 ± 0.01</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>4.2 ± 0.7</td>
<td>14.0 ± 1.2</td>
<td>2899 ± 151</td>
</tr>
</tbody>
</table>

Table 6.3 Enzyme activity in cv. Midas aged 25 days after anthesis.
Values are averages of 3 separate determinations ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>nmol CO₂ min⁻¹ tissue⁻¹</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transparent layer</td>
<td>Inner layers</td>
</tr>
<tr>
<td>RuBP carboxylase</td>
<td>n.d.</td>
<td>0*</td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>11.55 ± 2.25</td>
<td>6.05 ± 0.65</td>
</tr>
</tbody>
</table>

Table 6.4 Enzyme activity in Albino lemma aged 25 days after anthesis. Values are averages of 3 separate determinations except * which is the average of 2 determinations.
activity was found in the transparent layer of Albino lemma.

Invertase activity in the soluble fraction of tissue homogenates was assayed at pH 5.0 and pH 7.0. Activity was greater at pH 5.0 in the inner layers of both varieties and was about three times the activity at pH 7.0. In the transparent layer of cv. Midas, only activity at pH 5.0 was detected whereas low levels of invertase activity at both pH values were found in the transparent layer of Albino lemma.

6.3.2 RuBP Carboxylase and PEP Carboxylase

RuBP carboxylase and PEP carboxylase were assayed at pH 7.8 in the inner layers and transparent layer of the barleys cv. Midas and Albino lemma. The activities of the enzymes were expressed as nanomoles of CO₂ fixed per min per tissue. In addition, the activities of RuBP carboxylase and PEP carboxylase were expressed on a chlorophyll basis for the inner layers of cv. Midas. The assay for RuBP carboxylase was checked by preparing wheat flag leaf extracts which had a RuBP carboxylase activity of 2.9 ± 0.4 μmol CO₂ fixed/min/mg chlorophyll.

RuBP carboxylase activity was not detected in the transparent layer of cv. Midas (Table 6.3) and was not determined in the transparent layer of Albino lemma (Table 6.4). The enzyme was not active in the transparent layer of the wheat cv. Sicco (not shown). However, PEP carboxylase activity was detected in the transparent layers of both barley varieties. The activity in Albino lemma was about three times greater than that in cv. Midas. PEP carboxylase activity in the transparent
The inner layers of both barleys had PEP carboxylase activity with the activity in cv. Midas about twice that in Albino lemma. Cv. Midas also had a low level of RuBP carboxylase activity.

6.4 Discussion

Both sucrose synthase and invertase can hydrolyse sucrose and produce metabolites which may be used for respiration and starch synthesis. In the two barley cultivars used in this work, soluble invertase at pH 5.0 was found in the transparent layer and inner layers of the barley caryopsis. Although neutral invertase has been detected in low concentrations in young sugar cane, and in greater concentration in older tissues (Sacher et al., 1963), invertase activity in barley at pH 7.0 is not thought to be due to a second enzyme as soluble invertase activity in the wheat pericarp is due to an enzyme which has a pH optimum at 5.5 and is less active at pH 7.0 (H.L. Riffkun, personal communication).

Invertase activity in the placento-chalazal and pedicel tissues of maize kernels has been linked with the hydrolysis of sucrose prior to entry of the sugar into the endosperm tissue (Shannon and Dougherty, 1972). A soluble acid invertase which was active during early grain-filling declined as the grain matured and insoluble acid invertase activity increased. Shannon and Dougherty (1972) suggested that sucrose hydrolysis could be an integral step in the transfer of sucrose out of the phloem or into the starchy endosperm. This does not appear to be the case.
in soybean (Thorne, 1980) or in wheat (Jenner, 1974; Sakri and Shannon, 1975). Recent work by Chevalier and Lingle (1983) showed that sucrose is not hydrolysed as it moves from the phloem to the endosperm of barley or wheat. In addition, they measured insoluble invertase at pH 5.5 in the transparent layer and the inner pericarp of barley and found activity in the transparent layer until 8 days after pollination and again after 15 days. The activity in the inner pericarp was low. This does not agree with measured levels of soluble invertase activity in the inner layers of barleys cv. Midas and Albino lemma. However, Chevalier and Lingle (1983) measured insoluble invertase activity and also dissected out and discarded the crease region. In this work the crease tissue was included in the homogenate of the inner layers. It may be that invertase is associated with the vascular strand or surrounding tissues at the crease.

Sucrose synthase activity was not found in the transparent layer of cv. Midas and was low in Albino lemma 25 days after anthesis. Sucrose hydrolysis in the transparent layer must therefore be catalysed by invertase. These results agree with Chevalier and Lingle (1983) who found invertase activity was high in the transparent layer of wheat and sucrose synthase activity was minimal. However, these authors concluded that invertase, though present in this tissue, was inactive as significant levels of free sucrose and little free glucose or fructose were found in this tissue. Whether this is also the case in the barley cultivars used in this study is unknown as sugar levels in the tissues were not determined.

Sucrose synthase activity in the inner layers of Albino lemma
was greater than that in cv. Midas. As Albino lemma has no photosynthetic ability the major source of sucrose for the caryopsis is probably the stem, leaves and glumes. Sucrose entering the caryopsis via the vascular strand could be hydrolysed in the cross cells by sucrose synthase to provide substrates for starch synthesis.

The cross cells of the pericarp of barley and wheat have been shown to contain a number of enzymes characteristically associated with the pathways of C$_3$ and C$_4$ photosynthesis (Duffus and Rosie, 1973; Nutbeam, 1978; Wirth et al., 1977). The first-formed product of photosynthesis in immature barley cross cells has been identified as the C$_4$ dicarboxylic acid malate. As with other photosynthetic tissues however, sucrose is the major end product of CO$_2$ fixation (Nutbeam and Duffus, 1976). Thus the present results, in which the presence of RuBP and PEP carboxylase activity is reported in the cross cells of barley cv. Midas extend and confirm the previous findings. PEP carboxylase activity in the photosynthetic tissue of legume pods has been linked to the refixation of respired CO$_2$ (Hedley et al., 1975; Atkins et al., 1977) and a similar role has been postulated for PEP carboxylase activity in cereal pericarps (Duffus and Rosie, 1973). However, the evidence for CO$_2$ refixation by cross cell photosynthesis is not conclusive (Chapter 5). Therefore the role of PEP carboxylase in cereal cross cells may be other than the fixation of respiratory CO$_2$. Atmospheric CO$_2$ could be fixed by PEP carboxylase and incorporated into oxaloacetic acid/malate. Following decarboxylation, perhaps by malic enzyme, the released CO$_2$ might then be incorporated into triose phos-
phates via RuBP carboxylase and the enzymes of the photosynthetic carbon reduction cycle.

Alternatively, the fate of oxaloacetic acid/malate could be the tricarboxylic acid cycle whereupon the intermediates can be converted to sucrose via gluconeogenesis. The cross cells may therefore synthesise carbohydrates via the TCA cycle and gluconeogenesis. A similar function for PEP carboxylase in rice grains has also been postulated (Kundu et al., 1978). Thus it was suggested that its principal role is in the supply of dicarboxylic acids rather than the refixation of CO$_2$. Additionally, the products of PEP carboxylase activity may act as substrates for amino acid formation following transamination of TCA cycle intermediates. Transaminases have been detected in the wheat pericarp (Wirth et al., 1977) but no evidence has so far been presented which confirms the conversion of oxaloacetic acid/malate derived from PEP to amino acids and polypeptides.

Presumably cross cell PEP carboxylase fixes atmospheric CO$_2$ which has diffused through the transparent layer to the cross cells or entered via the stomata at the brush end of the grain. In C$_4$ plants PEP carboxylase is localized in the cytosol of the mesophyll cells (Hatch and Kagawa, 1973) and RuBP carboxylase is found in the chloroplasts of the bundle sheath (Chollet and Ogren, 1973). No such cell types are present in the cereal grain pericarp. PEP carboxylase and RuBP carboxylase activity are both present in the cross cells of the pericarp of barley (Duffus and Rosie, 1973) and it appears that there is no inter-
cellular separation of CO₂ fixation by the carboxylases.

The transparent layers of both barleys have PEP carboxylase activity. This tissue, which is composed solely of parenchymatous cells of the outer pericarp, has been shown to be capable of fixing ¹⁴CO₂ into soluble and insoluble material in both the light and dark (Chapter 3). The labelled material could also arise from cross cell ¹⁴CO₂ assimilation followed by rapid transfer of products to the transparent layer. However, since isolated transparent layers can fix ¹⁴CO₂ (Chapter 3) it seems likely that PEP carboxylase activity is responsible for the ¹⁴C-labelled material found in this tissue. The fate of some of the ¹⁴C-labelled products is starch (Chapter 4) which indicates that these cells contain starch synthesising enzymes during early grain development.

PEP carboxylase activity has also been detected in the non-photosynthetic tissues of cereals including the endosperm and embryo of barley (Nutbeam, 1978) and the endosperm and embryo of rice (Kundu et al., 1978). The role of the enzyme in these tissues is most likely to be in the synthesis of dicarboxylic acids as, during grain filling, metabolic activity in these tissues is high and intermediates of the TCA cycle are required for a range of biosynthetic reactions. Mitochondria have been shown to be present in the developing endosperm of barley (Duffus and Cochrane, 1982).

Previously, it has been shown that RuBP carboxylase is active in barley cross cells (Duffus and Rosie, 1973; Nutbeam, 1978) and wheat cross cells (Wirth et al., 1977). This was confirmed
here for the barley cv. Midas but interestingly no activity could be detected in the cross cells of *Albino lemma*. Electronmicrographs of the cells from *Albino lemma* show that they contain plastid-like organelles (M.P. Cochrane, personal communication) in which starch granules are present. These plastids are not amyloplasts but must contain the enzymes of starch synthesis. Nevertheless, in spite of having no chlorophyll or RuBP carboxylase activity in the plastids, this tissue is capable of fixing CO$_2$ both in the light and dark. The source of energy required to drive the biosynthetic reactions is presumably derived from other tissues of the plant.
This work has described some physiological and biochemical functions of the immature cereal pericarp. The contribution made by the pericarp to grain filling was investigated here by comparing the properties of a barley variety with green cross cells with those of a variety lacking chlorophyll in the cross cells.

Photosynthesis has been observed in the cross cells of barley and wheat and detached caryopses can fix atmospheric carbon dioxide. However, experiments with intact caryopses and caryopses minus the transparent layer show that the transparent layer is a barrier to CO₂. Photosynthate produced in the pericarp is translocated to the endosperm where it is incorporated into insoluble material. The mutant barley fixed atmospheric CO₂ but little assimilate was found in the endosperm.

Insoluble material is also synthesised in the transparent layer and the cross cells in response to CO₂ fixation. Starch is present in these tissues from anthesis until about midway through the grain filling period. In the cross cells starch seems to be turned over and could therefore be a temporary energy store for the developing grain. The products of starch degradation could also act as primers for starch synthesis. The cross cells seem to be similar to leaf cells where starch is rapidly synthesised and degraded. Whether starch in the cross cells persists during prolonged darkness is unknown. The enzymes of starch synthesis have not been studied but
degradative enzymes have been identified in the pericarp.

The route whereby assimilates produced in the pericarp reach the endosperm has been tentatively identified. It seems that the assimilates move round the caryopsis in the outer tissues to the crease region where they can enter the endosperm via the nucellar projection and the crease aleurone. If an experimental method could be used whereby soluble assimilates were retained in the tissues during processing for microautoradiography, stronger evidence for the route would be obtained. Freeze-substitution or freeze-sectioning are techniques which have been used successfully elsewhere. The movement of soluble material, probably sucrose, could thus be followed in the grain by giving the grain short pulses of $^{14}\text{CO}_2$ followed by chase periods in non-radioactive CO$_2$. Small areas of the grain could also be allowed to photosynthesise in $^{14}\text{CO}_2$ and the translocation of assimilate to the remainder of the grain observed. High resolution microautoradiographs might also yield information about apoplastic and symplastic movement in grain tissues.

The results of $^{14}\text{C}$-sucrose uptake by detached barley ears showed that more labelled insoluble material was recovered after culture in the light than in the dark. It seems that oxygen produced by pericarp photosynthesis stimulates starch synthesis in the endosperm. Oxygen is required for respiration, especially in the endosperm where starch is synthesised from a few days after anthesis until about fifty days after anthesis. The close proximity of the cross cells to the endosperm ensures
that oxygen and assimilates from these cells do not have a great distance to travel. Since oxygen is scarcely soluble in cuticular material, the main escape route for oxygen produced by the cross cells is through the stomata. However, these are few in number and have not as yet been shown to be functional. Cochrane and Duffus (1979) have suggested that the oxygen stays in the grain in the air space between the transparent layer and the cross cells and can pass into the endosperm via the vascular bundle. As yet the gases in the air space have not been analysed. It would be useful to establish the composition of the gas when the caryopsis is photosynthetically active. Oxygen may be involved in regulation of the activity of the grain and the photosynthetic production of oxygen may be an important part of the pericarp's contribution to grain filling.

Cross cell photosynthesis cannot be the sole source of oxygen for respiration as the mutant barley grows well and has a large well filled grain at maturity. The absence of chlorophyll in its cross cells does not seem to be detrimental to its grain filling capacity. Sources of oxygen for the grain could be the atmosphere via the stomata or other plant parts which would include the awn and the tip of the palea both of which are green for most of the grain filling period.

Substrates and energy for starch synthesis in the pericarp of the mutant are presumably derived from elsewhere in the plant. The presence of sucrose synthase and invertase in the transparent layer and cross cells/crease supports this hypothesis as these enzymes could metabolise translocated
sucrose. The products could then be used for respiration and starch synthesis.

It seems that pericarp photosynthesis has a role to play in reducing carbon losses from the grain. Incubation in the light reduced the loss of $^{14}C$ from radioactive caryopses and is presumably a result of the refixation of respired CO$_2$ by the pericarp. On the basis of this evidence and the activity of PEP carboxylase, the presence of which many workers have related to the refixation of respired CO$_2$, it is likely that the pericarp can reduce respiratory carbon losses. This work could be extended by investigating the CO$_2$ and O$_2$ requirements of the grain and whether the husk has a role to play in refixation of respired CO$_2$.

Whilst the pericarp has been shown to be capable of making a contribution to grain filling in vitro, the extent and importance of this contribution under normal environmental conditions is unknown. Comparisons with the mutant barley are of interest but little is known of its agronomic characteristics. If more was known about its properties, including any special adaptations it may have, it should be possible to reach firm conclusions about the role of the pericarp in grain filling.
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Monograph No. 12

REGULATION OF SOURCES AND SINKS IN CROP PLANTS

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PHOTOSYNTHESIS IN THE IMMATURE CEREAL PERICARP IN RELATION TO GRAIN GROWTH

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Summary. Some properties of the immature cereal pericarp are described. Significant levels of phosphoenolpyruvate carboxylase (PEPC) activity were present in the pericarps of barley, wheat and oats. This activity in general, was higher than that observed in leaves of barley, maize and Sedum. Rates of light-dependent carbon dioxide fixation in barley caryopses were significantly higher than those found in the leaf, when measured on a chlorophyll basis. Rates were low in comparison to those observed in maize and Sedum leaves. Evidence is presented which suggests that the products of carbon dioxide fixation in the pericarp can be translocated to the endosperm and incorporated into starch. It is further shown, using 14C-labelled caryopses, that the cross cells of the pericarp may be capable of refixing respired carbon dioxide in the light. The role of oxygen, evolved during pericarp photosynthesis, is discussed in relation to its possible effect on grain-filling.

INTRODUCTION

Photosynthesis and grain-filling

Until recently it was generally agreed that the greater part of the dry matter entering the developing cereal grain is derived from photosynthesis occurring after ear emergence (1, 2). There is now good evidence however, that some of the carbon found in mature grains is derived from assimilates formed before anthesis (3). The relative contributions of pre- and post-anthesis assimilate to grain-filling may depend on environmental conditions. For example, if photosynthesis after anthesis is limited by drought, persistent cloud or attacks by pathogens, then the plant may compensate by translocating assimilates derived from pre-anthesis photosynthesis to the
developing grain (4). It has been shown that the contribution to grain-filling of carbohydrate stored in the stem before anthesis may be as much as 30 per cent of final grain weight (5). Nevertheless, assimilates derived from photosynthesis in the ear itself, the flag leaf and the stem below the ear are considered to make a major contribution to grain-filling. Many investigations have demonstrated the importance of ear photosynthesis to yield. In barley for example, it has been estimated that assimilates produced in the ear may contribute as much as 76 per cent of grain dry weight (2) and Birecka et al. (6) have suggested that the ear contribution to total photosynthetic activity of barley could be up to 48 per cent. The proportion of grain dry weight met by ear photosynthesis depends on cultivar, environmental conditions and method of estimation (4,7). For example, in awned cultivars of wheat (7), ear photosynthesis contributed up to 76 per cent of total grain requirements during early grain growth but this fell to 26 per cent during the period of most rapid growth. In barley cv. Proctor the contribution of ear photosynthesis to final grain weight was found to be 13 per cent (5).

All green parts of the inflorescence can contribute to ear photosynthesis (8). Photosynthesis by developing wheat caryopses has been shown to account for 33-42 per cent of gross ear photosynthesis. Rates of photosynthesis were nearly maximal at the light intensity measured inside the glumes and almost balanced the loss of carbon dioxide by dark respiration (7).

The chloroplast-containing cells of the immature pericarp are the site of caryopsis photosynthesis. However, much speculation surrounds the possible role of pericarp photosynthesis in grain-filling. It is not clear for example whether the carbon dioxide fixed is derived from the atmosphere or from endosperm respiratory processes. The fate of any evolved oxygen is unknown.

Developmental morphology of the pericarp

Morphological studies of the outer layers of developing caryopses have led to an increased understanding of the possible role of the pericarp during grain-filling (9). Following fertilisation, the ovary wall becomes differentiated into the various tissues of the pericarp (Fig 1). The inner epidermal cells, known as tube cells, elongate in the long axis of the grain and become widely separated as the caryopses develops. The cells surrounding these elongate in a plane at right angles to the long axis of the grain and are known as cross cells. These form a continuous band, which is two to three cells thick in barley and one cell thick in wheat, rye, oats and rice. The cross cells contain chloroplasts, as do the thick wedges of
Fig. 1. Transverse section of the outer layers of the flank of a 30-'day' caryopsis of barley cv. Midas. The tissue was taken from the middle of the grain, fixed in glutaraldehyde and embedded in epoxy resin. The section, 2 μm thick, was stained in toluidine blue, pH 9.5. PE, pericarp epidermis; TL, transparent layer; CC, cross cells; T, testa; A, aleurone; SE, starchy endosperm. Bar represents 100μm. (Reproduced by permission of Dr M.P. Cochrane)

parenchymatous cells on either side of the pericarp vascular bundle. It is these chloroplasts which are responsible for caryopsis photosynthesis.

The outer part of the pericarp is a layer of non-chlorenchymatous cells. The outer epidermis has a cuticular layer on the outer wall and stomata are present on the ventral side near the stylar end. In barley (9) there are up to 10 stomata and in wheat there are more than 30 (10). The starch granules, present in the cells of the outer layer, increase rapidly in size following fertilization. Granule hydrolysis commences in the early stages of grain development but starch persists until just after the caryopsis has reached maximum length (11). With the disappearance of the starch granules, the outer layer becomes transparent and by 25 'days' post-anthesis can be two to six cells thick. Between 14 and 21 'days' after anthesis i.e. before maximum caryopsis length is achieved, the cells of the outer layer adjacent to the cross cells, degenerate leaving an air space which remains until the later stages of endosperm expansion. It appears, from morphological studies of immature cereal caryopses (9), that carbon dioxide can reach the cross cells from the atmosphere following diffusion through stomata and the surface of the pericarp epidermis, and from the endosperm by diffusion through the cuticular layers of the testa.
and nucellus. Oxygen, evolved in cross cell photosynthesis may accumulate in the gap outside the cross cells and eventually enter the endosperm via the vascular bundle.

Carbohydrate metabolism in the immature pericarp.

The immature wheat and barley pericarp contains a number of enzymes characteristically associated with the pathways of C3- and C4-photosynthesis (12,13). These include phosphoenolpyruvate carboxylase (PEPC), ribulose-1, 5-bisphosphate carboxylase and possibly pyruvate orthophosphate dikinase (12,14). The first-formed product of photosynthesis in immature barley cross cells is the C4-dicarboxylic acid malate. As with other photosynthetic tissues however, sucrose is the major end-product of carbon dioxide fixation (15).

In the work which follows, some of the biochemical properties of the immature cereal pericarp are described and its possible contribution to grain-filling discussed.

MATERIALS AND METHODS

Plant Material.

Plants were grown under glasshouse conditions with natural daylength extended to 18 h using mercury vapour lamps. Grains were taken from the middle of ears and their age estimated subjectively in 'days after anthesis' using a table of morphological stages similar to that described by Baxter (16).

Phosphoenolpyruvate carboxylase (PEPC) activity.

Tissue homogenates were prepared in a medium containing 0.33 M sorbitol, 50 M tricine-KOH buffer, pH 7.5, 1mM MnCl₂, 1mM MgCl₂ and 10mM cysteine. After filtration through four layers of muslin, 0.2 ml of the filtrate was placed in small test tubes. To each tube was then added 0.03 ml of 0.75 M sodium (¹⁴C) bicarbonate (specific activity 12.2 M Bq/mmol) and 0.03 ml of 80 mM sodium glutamate in the grinding medium. After equilibrating at 30°C for 5 min, the reaction was initiated by the addition of 0.03 ml of 33 mM phosphoenolpyruvate in the grinding medium. Samples (0.1 ml) were withdrawn immediately and at suitable times between 90 s and 5 min. The reaction was stopped by placing in 0.5 ml of 5% (w/v) trichloroacetic acid in ethanol. The ethanolic mixtures were then heated in a water bath at 70°C for 30 min to drive off unfixed ¹⁴CO₂. After centrifugation at 5,000 x g for 10 min the supernatants were counted for radioactivity using a Beckman scintillation counter.
Carbon dioxide fixation in intact tissues.

The rates of carbon dioxide fixation were measured by placing the tissues on glass fibre discs in a perspex chamber (volume 11 cm$^3$) and allowing them to photosynthesise in $^{14}$CO$_2$ (0.1% carbon dioxide) as described previously (15,17).

Transfer of products of carbon dioxide fixation.

Detached caryopses of barley cv Julia and Albino lemma and of wheat cv Maris Dove from which the transparent layer of the pericarp had been removed, were incubated in $^{14}$CO$_2$ for 10 min in the light as described above. The cross cells and testa were then removed and discarded. The endosperm was then homogenised in 3 ml of 1% (w/v) sodium fluoride solution. Insoluble material was removed by centrifugation (10,000 g for 10 min) and washed twice further with sodium fluoride solution. After each washing, samples (0.5 ml) of the supernatant were counted for radioactivity. The solid material was then made up to 3 ml with water and heated in a boiling water bath for 10 min to solubilise starch. Insoluble material was removed by centrifugation (10,000 g for 10 min). A sample of the boiling water extract was counted for radioactivity. The pellet was resuspended in water, heat treated, centrifuged and the supernatant counted for radioactivity.

Incorporation of $^{14}$CO$_2$ into immature barley caryopses.

Detached, intact caryopses were incubated in a perspex chamber as described above. After exposure to $^{14}$CO$_2$ for 15 min in the light (710 μE m$^{-2}$ s$^{-1}$) they were removed from the chamber and placed in open dishes containing fresh filter paper and buffered medium. They were then maintained for 3 h either in light or dark at 21°C. The caryopses were washed in distilled water and the transparent layer, cross cells and the endosperm (including the embryo) were separated. The tissues were homogenised in boiling 80% (v/v) ethanol with hand-held all-glass tissue grinders. After washing with two aliquots of hot ethanol, the washings plus ethanol-insoluble material were centrifuged at 700 g for 2 min. The supernatant contains organic acids, sugars, sugar phosphates and possibly some protein (ethanol-soluble fraction). The ethanol-insoluble fraction was mixed with 5 ml of distilled water and left to extract at room temperature for 24 h. This was centrifuged at 700 g for 2 min. The supernatant contains soluble protein and oligosaccharides (water-soluble fraction). The water-insoluble residue was washed twice with 4 ml of distilled water and boiled for 10 min to solubilise starch. Acid hydrolysis (2½ h in 2M HCl at 100°C) was also used to hydrolyse starch. The solutions were tested with I$_2$/KI to ensure that all starch had been hydrolysed. All fractions were counted for radioactivity using liquid scintillation and measurements were corrected to
Significant levels of PEPC activity are present in the embryo, transparent layer, cross cells and endosperm of immature barley caryopses (Table 1). It is possible that this enzyme serves the dual function of 'topping-up' Krebs cycle intermediates and trapping carbon dioxide released in respiration. The anaplerotic function of PEPC is probably most important in cells synthesising protein, since a number of Krebs cycle intermediates can be transaminated to form amino acids.

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total Activity (pkats)</th>
<th>Relative Activity (nkats (g Fresh weight)^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo</td>
<td>18 ± 1.2</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Transparent Layer</td>
<td>22 ± 2.3</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>Cross Cell Layer</td>
<td>111 ± 6</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Endosperm</td>
<td>150 ± 33</td>
<td>2.8 ± 0.8</td>
</tr>
</tbody>
</table>

*Figures are means of four determinations ± standard deviation.
†The transparent layer dehydrates very quickly following excision. The 'true' figure is therefore lower than the one shown here.

Of particular interest is the relatively high activity of PEPC in the pericarps of barley, wheat and oats (Table 2), compared to that found in leaves of barley, maize and Sedum on a chlorophyll content basis. These results also show that the mutant Albino lemma, which has no chlorophyll in the cross cells, has a low but significant PEPC activity. This is similar to the activity found in cv Scottish Bere, a low yielding 6-row barley. Since chlorophyll contents of green tissues are variable, it is possible that these results may simply reflect differences in chlorophyll contents of the tissues examined. However, the relatively high activities of PEPC in barley cross cells correlate well with the observation (15) that the first-formed product of photosynthesis in this tissue is the C4-dicarboxylic acid malate.

The rate of light-dependent carbon dioxide fixation in isolated immature barley pericarps is low when compared with the rates observed under the same conditions for maize and Sedum leaves (Table 3). It is noteworthy that the rates of photosynthesis in barley caryopses (with the outer trans-
Table 2

Phosphoenolpyruvate carboxylase activity in different plant tissues

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>ENZYME ACTIVITY (nkats mg⁻¹chlorophyll)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERICARP CROSS CELLS</td>
<td></td>
</tr>
<tr>
<td>BARLEY²</td>
<td></td>
</tr>
<tr>
<td>cv Julia</td>
<td>30 ± 8</td>
</tr>
<tr>
<td>cv Santor</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>cv Scottish Bere</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>WHEAT</td>
<td></td>
</tr>
<tr>
<td>cv Maris Dove</td>
<td>185 ± 22</td>
</tr>
<tr>
<td>OATS</td>
<td></td>
</tr>
<tr>
<td>cv Astor</td>
<td>125 ± 6</td>
</tr>
<tr>
<td>LEAVES</td>
<td></td>
</tr>
<tr>
<td>BARLEY</td>
<td></td>
</tr>
<tr>
<td>cv Julia</td>
<td>5 ± 1.6</td>
</tr>
<tr>
<td>MAIZE</td>
<td></td>
</tr>
<tr>
<td>cv Golden Bantam</td>
<td>47 ± 22</td>
</tr>
<tr>
<td>Sedum spectabile</td>
<td>11 ± 1.6</td>
</tr>
</tbody>
</table>

1 All figures are means of four determinations ± standard deviations.
2 Pericarps from barley cv Julia were taken from caryopses 25 'days' after anthesis. Caryopses from the other cereals were sampled at a similar stage of development. Leaves were sampled when fully expanded.
3 Enzyme activity in cross cells from the chlorophyll-less mutant Albino lemma was estimated using the intact cross cell layer. Activity was 24 ± 0.8 nkats per tissue. This compares with a value for cv Scottish Bere of 26 ± 0.5 nkats per tissue.

parent layer removed) were significantly greater than those observed in the leaves. This is a further indication that the immature pericarp cross cells may have some unusual physiological and biochemical characteristics. These results confirm that carbon dioxide fixation by barley caryopses is a light-dependent process although there is a small, but significant, amount of carbon dioxide fixed in the dark. The low rates of fixation observed with isolated pericarps may be a consequence of tissue damage incurred during dissection. Alternatively, full rates of carbon dioxide fixation may only be achieved in the presence of a sink for the newly synthesized photosynthetic assimilates. Removal of the sink might lead to the accumulation of intermediates of photosynthesis in the pericarp cross cells and hence result in a reduction in the rate of carbon dioxide fixation.
Table 3

Rates of carbon dioxide fixation by different tissues

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>CONDITION</th>
<th>RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n mol CO₂ mg⁻¹ chlorophyll min⁻¹</td>
</tr>
<tr>
<td>Barley (cv Julia²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>light</td>
<td>17 ± 9</td>
</tr>
<tr>
<td>Isolated pericarp</td>
<td>light</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>Caryopsis</td>
<td>light</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>Caryopsis</td>
<td>dark</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>Maize (cv Golden Bantam)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>light</td>
<td>127 ± 19</td>
</tr>
<tr>
<td>Sedum spectabile</td>
<td>light</td>
<td>130 ± 80</td>
</tr>
<tr>
<td>Pea (cv Meteor)</td>
<td>light</td>
<td>34 ± 10</td>
</tr>
</tbody>
</table>

¹Figures are the mean of five determinations ± standard deviations.
²Pericarps from barley cv Julia were taken from caryopses 25-30 days after anthesis. Wheat caryopses were sampled at a similar stage of development. Leaves were sampled when fully expanded.
³Outer transparent layer removed.

FATE OF THE PRODUCTS OF CARBON DIOXIDE FIXATION

Preliminary experiments, in which the transparent layer was removed from caryopses before incubation in ¹⁴CO₂, suggested that the products of cross cell photosynthesis could be transferred to the endosperm and incorporated into starch (Table 4). The greatest amount of label was recovered from wheat (cv Maris Dove) endosperm. This correlates with the higher levels of PEPC activity observed in this cultivar compared to barley cv Julia (Table 2). Interestingly there was a small amount of label found in the starch fraction from the endosperm of the mutant barley Albino lemma. Incorporation is presumably a result of non-photosynthetic carbon dioxide fixation catalysed by PEPC (Table 2).

In further experiments, radiocarbon-labelling studies have been used to follow the incorporation of externally-supplied CO₂ into the different tissues of the immature barley caryopsis. The distribution of label between the ethanol-soluble and starch fractions of the transparent layer, cross cells (including the testa) and the endosperm (including the embryo), following exposure of intact caryopses to ¹⁴CO₂ is shown in Table 5. Amounts of label in the water-soluble fraction are not shown. After illuminated incubation (15 min) of the caryopses, the greater part of the
Transfer of the products of carbon dioxide fixation from the cross cells to the endosperm

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Amount of $^{14}$C recovered in endosperm starch$^2$ (pmol caryopsis$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley Albino lemma</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Barley cv Julia</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Wheat cv Maris Dove</td>
<td>10.1 ± 2.3</td>
</tr>
</tbody>
</table>

$^1$Caryopses from barley cv Julia were sampled between 25 and 30 days after anthesis. Those from wheat and barley Albino lemma were sampled at the stage of development most closely corresponding to that of barley cv Julia. The transparent layers were removed before incubation in the light for 10 min. After removal of cross cells and testa, a starch fraction from the endosperm was counted for radioactivity.

$^2$Figures are the mean of three determinations ± standard deviation.

Recovery of $^{14}$C was found in the ethanol-soluble fraction of the cross cell layer. Significant levels of radioactivity were also found in the ethanol-soluble fraction of the transparent layer and the endosperm.

The starch fraction from all three tissues also contained radiolabel although the amounts in the endosperm were low. Greater amounts of label were detected in endosperm starch if caryopses were incubated under similar conditions with the transparent layer removed (results not shown). The amount of $^{14}$CO$_2$ fixed by intact caryopses in the dark was low (results not shown) and label was found only in the transparent layer of the pericarp. This was presumably a result of carboxylation catalysed by PEPC (Table 1). These results confirm that externally-supplied carbon dioxide can be fixed in the light by intact caryopses. They further suggest that some of the products can be translocated to the endosperm and incorporated into starch.

Other results (P.A. Scragg in preparation), indicate that intact caryopses of Albino lemma are capable of light-dependent carbon dioxide fixation. The rates of fixation are between 10 and 50 per cent of those observed in 'normal' cultivars. This may be a result of light-triggered stomatal opening leading to an increased uptake of atmospheric carbon dioxide. Any effect on grain-filling would then be a consequence of non-photosynthetic carboxylation catalysed by PEPC. In the dark the stomata may be closed, thus reducing the amount of carbon dioxide available. It is also possible that atmospheric oxygen may enter the caryopsis via the open stomata, thereby compensating for the lack of oxygen evolution in photosynthesis (q.v.).
Table 5

Incorporation of $^{14}$CO$_2$ into barley (cv Midas) caryopses 25 'days' after anthesis$^1$

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ethanol-soluble fraction dpm</th>
<th>Starch fraction dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 15 min in $^{14}$CO$_2$ (light)$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transparent layer</td>
<td>$4650 \pm 3420$</td>
<td>$150 \pm 70$</td>
</tr>
<tr>
<td>cross cell layer</td>
<td>$7650 \pm 4900$</td>
<td>$2250 \pm 810$</td>
</tr>
<tr>
<td>endosperm</td>
<td>$11930 \pm 1330$</td>
<td>$110 \pm 100$</td>
</tr>
<tr>
<td>2. 3h chase in $^{12}$CO$_2$ (light)$^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transparent layer</td>
<td>$4990 \pm 790$</td>
<td>$250 \pm 50$</td>
</tr>
<tr>
<td>cross cell layer</td>
<td>$3360 \pm 1030$</td>
<td>$1480 \pm 560$</td>
</tr>
<tr>
<td>endosperm</td>
<td>$3210 \pm 920$</td>
<td>$440 \pm 140$</td>
</tr>
<tr>
<td>3. 3h chase in $^{12}$CO$_2$ (dark)$^4$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transparent layer</td>
<td>$1600 \pm 400$</td>
<td>$170 \pm 20$</td>
</tr>
<tr>
<td>cross cell layer</td>
<td>$1130 \pm 290$</td>
<td>$170 \pm 90$</td>
</tr>
<tr>
<td>endosperm</td>
<td>$1510 \pm 590$</td>
<td>$130 \pm 30$</td>
</tr>
</tbody>
</table>

$^1$Intact, detached caryopses were exposed to $^{14}$CO$_2$ for 15 min in the light. They were then maintained for 3 h either in light or dark. After washing, the different tissues were separated and counted for radioactivity (see Materials and Methods).

$^2$Figures are the mean of three separate determinations ± standard deviation.

$^3,4$Figures are the mean of four separate determinations ± standard deviations.

In order to examine the fate of radiolabelled compounds following photosynthesis, the distribution of radioactivity between the various fractions was determined following a 3 h chase in $^{12}$CO$_2$ (Table 5). Only a small proportion of the original label remained, suggesting that the respiration rate of immature caryopses must be high. Caryopses illuminated during the chase period retained twice as much radioactivity as the dark-treated caryopses. This is presumably a result of the refixation of respired $^{14}$CO$_2$, and indicates that illumination prevents some respiratory loss.

It seems likely that pericarp photosynthetic assimilates are the main substrate for grain respiration since by far the greatest proportion of $^{14}$C-label was lost from the cross-cell layer ethanol-soluble fraction. No loss of label from the transparent layer ethanol-soluble fraction was observed during the light chase. This indicates that the pool of intermediates remains saturated while those of the endosperm and cross cell...
layer are depleted. Radioactivity in the transparent layer and endosperm starch fractions increased during the light chase. This suggests that while starch may be turned over rapidly in the cross cells, the starch of the transparent layer and endosperm amyloplasts, once laid down, is not subsequently degraded and respired. Again, the results indicate that the products of cross cell photosynthesis can be translocated to the endosperm and incorporated into starch.

**OXYGEN**

It has been suggested that oxygen may be involved in the regulation of sink activity in the developing grain via its role in oxidative phosphorylation (18). The source of the oxygen is unknown. One possibility is that some at least is derived from photosynthesis in the pericarp cross cells (19,20). Another possibility is that it is produced from photosynthesis elsewhere in the plant and enters the endosperm in solution via the phloem stream. Oxygen could also enter the developing caryopsis from the atmosphere through the stomata of the pericarp epidermis or through stomata in other parts of the plant.

It has now been demonstrated (20) that light can stimulate the incorporation of U-\(^{14}\)C-sucrose into the starch of developing wheat kernels cultured in liquid medium. Whether this represented incorporation into endosperm starch or cross cell starch was not reported. This response to light was eliminated by removal of the green layer or by inhibition of its photosynthesis by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). When no oxygen, but a photosynthetic level of carbon dioxide was supplied above the culture medium, starch synthesis was virtually eliminated unless light was provided. These experiments suggest that photosynthetic generation of oxygen by the cross cells of the pericarp is a possible cause of the light response. In other experiments (21) using detached ears of wheat in liquid culture, it has been shown that there was a decrease in the rate of grain growth with shading at all levels of sucrose supplied. It was not possible to conclude however, whether irradiance influenced dry matter accumulation simply through a photosynthetic contribution, such as assimilate supply or oxygen, or by some other mechanism.

In immature detached barley caryopses 25 'days' after anthesis the amount of oxygen evolved in cross cell photosynthesis was sufficient to supply that taken up in respiration (19). In the caryopsis of the chlorophyll-less mutant *Albino lemma* on the other hand, there was always a net oxygen uptake in the light. Under glasshouse conditions, grain-filling in *Albino Lemma* falls well within the range observed for other barley cultivars and final grain weight is not significantly less.
It must be concluded that, at least in this cultivar, any oxygen required for sink activity is derived from sources other than cross cell photosynthesis. It may be that the mutant has some special adaptations which compensate for the lack of chlorophyll in the cross cells. That the cross cells are not the sole source of oxygen for grain-filling is indicated by the observation that pericarp photosynthesis in most cultivars continues for not more than the first two thirds of the maturation period (12). Grain-filling however continues in its absence, albeit at a reduced rate, and endosperms remain capable of respiratory activity, as measured by oxygen uptake, until a late stage of development (Dr B. Ahluwalia, personal communication).

Since oxygen is thought to be sparingly soluble in cuticular material and the epidermal stomata are few in number, it seems likely that most of the oxygen produced by cross cell photosynthesis remains within the caryopsis. It has been suggested (9) that the oxygen diffuses round the caryopsis in the gap between the green layer and the transparent layer, which forms when the pericarp-cells immediately outside the cross cells disintegrate, and enters the endosperm via the chalazal region and the nucellar projection at the crease. Carbon dioxide may be less insoluble in lipid material than oxygen and it is possible therefore that it may reach the cross cell layer from the endosperm by diffusion through the cuticular layers - or possibly from the atmosphere via the stomata and the pericarp epidermis. Thus in addition to its possible role in the fixation of carbon dioxide and the supply of carbohydrate precursors, the pericarp may have a role in the regulation of sink activity by controlling the oxygen supply.

CONCLUSIONS

To date there has been no direct evidence that photosynthesis in the cross cells of the pericarp makes any contribution to grain dry weight in intact cereal plants under field conditions. The results reported here suggest however that the pericarp is metabolically active and capable of making a small but significant contribution to grain-filling in vitro. Clearly in any assessment of the contribution of the pericarp to grain-filling, the results obtained with the barley Albino lemma, which has no chlorophyll in the cross cells, are of particular importance. However, until more is known of the agronomic and physiological characteristics, including any special adaptations, of Albino lemma, results with this mutant cannot be used in coming to a firm conclusion on the contribution of the pericarp to grain-filling. On the other hand, the results from in vitro culture systems do indicate that light has a positive effect on starch accumulation in the
endosperm of developing caryopses and that the effect is possibly mediated by triggering cross cell oxygen evolution and/or the fixation of carbon dioxide, derived either from the atmosphere or from internal respiratory processes.

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References


