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ROTHAMSTED RESEARCH

# Rothamsted Experimental Station Report for 1985

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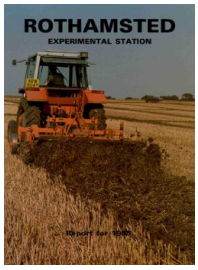


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### B. J. Miflin

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# MOLECULAR SCIENCES DIVISION

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## BIOCHEMISTRY DEPARTMENT

### Introduction

It is often said, particularly at gatherings of plant molecular biologists, that our ability to progress in the genetic manipulation of plants is limited by the failure of plant biochemists to provide sufficient information of plant metabolism. This is to suggest there is somehow a dichotomy between plant molecular biology and biochemistry. Such comments misunderstand the nature and the problems of plant biochemistry. It has been the philosophy of this Department that the whole range of techniques that make up genetic manipulation are just one part (albeit an increasing and extremely powerful part) in the armoury of approaches that may be used to attack the problems of biochemistry. Likewise, biochemistry is just one of several disciplines that must be brought to bear on problems of efficient crop production in which both yield and crop quality are vitally important.



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Thus to prosecute biochemistry effectively and rapidly it is necessary to develop and use the most powerful of techniques. This has led to very rapid progress in recent years and it is pleasing to be able to report further success in demonstrating the utility of this approach, both in terms of advancing scientific knowledge and in obtaining financial support for our work. This is particularly emphasized this year in our study of cereal proteins where a full range of techniques including protein separation and characterization, nuclear magnetic resonance and circular dichroism spectroscopy, DNA sequencing, and computer structural prediction have been brought to bear on understanding the role of wheat seed proteins in determining the functional properties of gluten. Our cereal protein programme has been linked to a study of factors affecting the deposition of proteins in the grain. Studies on the effect of crop nutrients and genetics on the composition of the grain have pointed to the importance of understanding the control of gene expression in this system. Current progress in this area is highly encouraging, but to really understand the process we have to develop techniques of gene transfer into cereals and cereal regeneration. Fortunately our cell biology programme has been as successful as any in this area and we are optimistic as to future progress.

A similar wide range of interactive approaches are being brought to bear on the pathway of carbon in photosynthesis and photorespiration, the uptake, assimilation and metabolism of nitrogen, the regulation of development and metabolism in potatoes, and the reaction of plants to viral infection. Selected results are reported below.

It is also gratifying to record that scientific progress has been accompanied by outside financial investment in the Department's programme. Currently, besides the basic grant-in-aid, we have support from the AFRC New Initiatives in plant science and food science, from a number of national and international funding agencies, including the EEC biomolecular engineering programme (two grants), and directly from industry. Furthermore, the funding promised via the Agricultural Genetics Company has now come to fruition and we are pleased that we have attracted four contracts from them during the current year. Nevertheless, the future could be clouded by the planned reorganization of the AFRC and it is imperative to avoid creating the false dichotomy referred to at the beginning of this introduction.

## Carbon metabolism

Some of the work formerly in the research area on 'Photosynthetic carbon and nitrogen metabolism' is included in this new research area together with research on ribulose bisphosphate carboxylase and on the enzymes of photosynthetic carbon assimilation. The catalytic mechanisms and structure of ribulose bisphosphate carboxylase are being investigated within the AFRC priority programme on photosynthesis. A specific search was made among the photorespiratory mutants for a lesion affecting carboxylase activity or activation that might help in studies of the enzyme. Also development of expression vectors containing the genes for the peptide subunits of a cyanobacterial carboxylase was begun. The objective is to undertake site specific *in vitro* mutagenesis to provide further information concerning the catalytic activities of the RuBP carboxylase/oxygenase and eventually to obtain an enzyme of $L_8S_8$ structure that has decreased oxygenase activity. Decreased photorespiration resulting from introduction of an improved carboxylase should increase productivity considerably. (Area coordinator: Keys; Bright, Burton, Cornelius, Gutteridge, Hall, Johnson, Kendall, Kettleborough, Millard, Parry, Phillips, Rintamaki, Turner, Wallsgrove)

**Photorespiratory mutants.** Some 50 mutant barley lines, sensitive to air with ambient $CO_2$ but able to grow in $CO_2$-enriched air (>0·5% $CO_2$), have been selected. Lesions in 24 of these lines have now been identified. Newly characterized classes include mutants deficient in phosphoglycollate phosphatase and in glutamine synthetase. A mutant with decreased



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content of RuBP carboxylase protein and activity was also identified. Since the last report, a further five mutants deficient in catalase, four in glutamate synthase (GOGAT) and one in 2-oxoglutarate uptake have been identified.

The mutant deficient in phosphoglycollate phosphatase (RPr84/90) accumulated 25% of assimilated $^{14}C$ in phosphoglycollate during five minute photosynthesis in air containing $^{14}CO_2$. Three proteins with phosphoglycollate phosphatase activity are present normally in barley and can be separated by gel electrophoresis; all three active proteins were absent from the mutant.

RPr84/29 is a mutant with decreased rates of photosynthesis in 1% and 21% $O_2$. It contains only 30% of the normal amount of RuBP carboxylase protein, although the properties of the purified enzyme were not different from those of the wild type.

RPr83/32, 84/12, 84/34 and 84/84 are deficient in glutamine synthetase activity. Separation of the isozymes by FPLC (fast protein liquid chromatography) confirmed that the deficiency was in the chloroplast form. Photosynthesis was inhibited more slowly than in GOGAT-deficient mutants on transfer to 21% $O_2$ containing 350 $\mu$l $CO_2$ $l^{-1}$ although ammonia accumulated in the leaves faster. Thus the rapid inhibition of photosynthesis in GOGAT mutants in air was probably not due to uncoupling of photophosphorylation caused by ammonia. Isolation of glutamine synthetase mutants has confirmed that the GS/GOGAT cycle is responsible for photorespiratory ammonia assimilation with the chloroplast glutamine synthetase having a major role.

**Cataiytic site of ribulose bisphosphate carboxylase.** Amino acid residues near the N-terminus of the large subunit polypeptides of higher plant ribulose bisphosphate carboxylase were shown to be essential for catalysis. Thus low concentrations of trypsin rapidly removed two peptides from the large subunit; the first had an $M_r$ of 1500. The catalytic site residues appear to be in the second peptide. Carboxylases from barley and tobacco were more resistant to proteolysis than the enzyme from wheat, spinach or maize. Proteolysis, sufficient to inactivate the enzyme, did not prevent the binding of the activating $CO_2$ molecule or the divalent metal cofactor. The ribulose bisphosphate binding site was also retained, since 2-carboxyarabinitol bisphosphate bound strongly to the partially hydrolysed enzyme when $CO_2$ and $Mg^{2+}$ were also bound.

**Regulation of carboxylase activity by anionic effectors.** In 1984 it was reported that orthophosphate increased the activity of RuBP carboxylase without increasing the amount of bound activating $CO_2$. Further experiments have shown that 6-phospho-D-gluconate, at or below equimolar amounts with the protein active sites, also increased activity without increasing the amount of bound activating $CO_2$. Above equimolar amounts the bound activating $CO_2$ was increased without further increase in activity. Preliminary experiments with other effectors, including NADPH, suggested that all could increase activity without increasing $CO_2$ bound at the activating site.

**Dark regulation of ribulose bisphosphate carboxylase.** In joint work with Dr J. C. Servaites (University of Dayton, Ohio, USA) a study was made of the taxonomic distribution of an inhibitor of ribulose bisphosphate carboxylase that becomes bound to the enzyme at night (Servaites, *Plant Physiology* (1985) **78,** 839–843; Vu, Allen & Bowes, *Plant Physiology* (1983) **73,** 729–734). The presence of the inhibitor was not obviously associated with any physiological, biochemical or taxonomic classification of the plant species examined. A convenient source of the inhibitor was found in the carboxylase protein extracted from potato leaves after a prolonged dark period. The compound is of low molecular weight, is phosphorylated and produces 50% inhibition of activity when incubated with the enzyme in the ratio of two moles of inhibitor per eight catalytic sites.



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**Expression of genes for ribulose bisphosphate carboxylase from *Synecoccocus*.** Cloned genes for the large and small subunit polypeptides for ribulose bisphosphate carboxylase of *Synecoccocus* can now be expressed at a satisfactory level in *E. coli*. The cloned genes, kindly donated by Dr A. A. Gatenby of the Plant Breeding Institute (PBI), Cambridge in the plasmid pLa2311, were isolated and transferred to pUC9. The genes in this plasmid pSynRES1 were satisfactorily expressed in *E. coli* as an active enzyme. Five litres of culture have provided some 35 mg of purified protein with a specific activity similar to that of the fully assembled $L_8S_8$ enzyme from *Synecoccocus*. About 50% of the L-polypeptide molecules are expressed in *E. coli* as a fusion product that originates from an extension of the N-terminus; 24 amino acids of the extension are from transcription of the 5′-flanking sequence and 10 from $\beta$-galactosidase. The extension prevents this L-subunit derivative from assembling correctly into a holoenzyme. The properly assembled active enzyme can be readily resolved from the less active aggregates by FPLC.

## Metabolic regulation

Knowledge of the pathways of amino acid biosynthesis and their regulation has been gathered slowly over the years. This has been applied, particularly to the production of nutritionally essential amino acids. Certain of our mutants with relaxed control on the synthesis of amino acids of the aspartate family have now been incorporated into a plant breeding programme for practical evaluation. Reactions of amino acid synthesis are good targets for herbicides. This is perhaps not surprising since the loss of ability to produce a given amino acid is one of the most frequently observed auxotrophic mutations. The synthesis of the branch chain amino acids is of particular interest because, firstly, a key enzyme (acetohydroxy acid synthase) is a target for two new classes of herbicides and second, a number of mutants auxotrophic for these amino acids are now known. We report studies of this pathway and also of continued work on the synthesis of the aspartate family of amino acids. (Area coordinator: Bright; de Bry, Franklin, Hill, Karp, Relton, Risiott, Turner, Wallsgrove)

### Branched chain amino acid synthesis

***Auxotrophic mutants.*** The biochemical lesions in six amino acid-requiring auxotrophic lines of *Nicotiana plumbaginifolia* (isolated by Dr I. Negrutiu at the Vrije Universiteit Brussels, Belgium) have been investigated. Characterization by feeding [$^{14}$C] and unlabelled substrates, and by enzyme analysis, has shown that three lines which require isoleucine for growth, are unable to synthesize 2-oxobutyrate *in vivo* and have no detectable threonine dehydratase activity. Three further lines require isoleucine plus valine, accumulate [$^{14}$C] dihydroxymethylvalerate when fed [$^{14}$C]-L-threonine, and contain no detectable dihydroxy-acid dehydratase.

The biochemical lesion in a *Datura innoxia* cell line (IV-1) that requires isoleucine and valine for growth, was investigated. The mutant cells were unable to synthesize isoleucine from [$^{14}$C] threonine, though both wild-type and mutant cultures converted [$^{14}$C] 2-oxomethylvalerate to isoleucine. Enzyme analysis has shown that IV-1 has no detectable activity of dihydroxyacid dehydratase, which is probably the cause of the amino acid requirement. Branched-chain amino acid aminotransferase activity in IV-1 was only 30% of that in the wild-type but the reason for this is not clear. The other enzymes of the pathway had essentially similar activities in both IV-1 and the wild-type line. These studies confirm that the branched chain amino acids are synthesized by a pathway in which four of the enzymes are bifunctional in that they catalyse reactions leading to both the synthesis of valine (and indirectly leucine) and its higher homologue isoleucine.



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***Acetohydroxyacid synthase.*** Acetohydroxyacid synthase has been extracted from leaves of three valine-resistant ($Val^r$) tobacco (*Nicotiana tabacum*) mutants (isolated by Dr J-P. Bourgin, Versailles), and compared to the wild-type enzyme. The enzyme from all three mutants is significantly less sensitive to inhibition by leucine and valine than that from the wild-type. Two of the mutants, $Val^r$-1 and $Val^r$-6, have very similar enzymes which, under all conditions, are inhibited by less than half that of the wild-type. The other mutant, $Val^r$-7, has an enzyme that only displays significantly different characteristics to the wild-type at high pyruvate or inhibitor concentrations. The enzyme from $Val^r$-7 also has an apparent Km for pyruvate threefold greater than that from the wild-type and the other mutants. The sulphonylurea herbicides strongly inhibit the enzyme from all the lines, though the concentrations required for half-maximal inhibition of enzyme from $Val^r$-1 and $Val^r$-6 were higher than for $Val^r$-7 or the wild-type. No evidence was found for multiple isoforms of acetohydroxyacid synthase. Thus the valine-resistance of these mutant lines may be the result of two different mutations affecting a single enzyme.

**Aspartate pathway.** In a collaborative study with the University of Lancaster considerable advances have been made in the purification of aspartate kinase. The aim is to devise a technique for complete purification of this important enzyme, probably including the use of monoclonal antibodies. Studies have continued on potential inhibitors of methionine biosynthesis; the most interesting finding is that plants have an active racemase converting D-methionine into the L-form. Under conditions in which it has been established previously that L-methionine is required for plant growth, D-methionine was almost as effective in relieving inhibition.

## Plant cell biology

The aim of this programme is to develop efficient systems of regenerating arable crop plants (wheat, barley, potato, rape, sugar beet) from isolated protoplasts, cells or tissues by *in vitro* culture. These systems are being applied to investigate methods to transfer or introduce useful genetic information into and between plant cells, with the regeneration of intact modified plants. These techniques are essential, not only to produce improved crop plants but also for the analysis of plant metabolism in order to identify key reactions and processes limiting crop production. Methods of gene transfer studied include using *Agrobacterium* and its engineered derivatives as vectors, protoplast fusion and direct gene transfer into protoplasts and tissues. The changes induced by the culture process itself are being evaluated to determine their origins, whether they can be controlled, and possible applications to crop improvement.

Clearly work on cell biology and tissue culture of arable crop plants is closely integrated with other aspects of research in the Department, to mutual benefit. Thus biochemical and molecular techniques are being used as tools in cell biological work, and the production of tissues and plants of important cultivars and breeding lines containing inserted genes is vital for assessment of molecular constructs, modifications to key biochemical pathways, and the future application of molecular techniques. (Area coordinator: M. G. K. Jones; Atkinson, Bains, Bright, de Both, Cooper-Bland, Darrell, Eady, Fish, Foulger, Gerentes, Karp, Leatham, Maddock, Ooms, Potter, Risiott, Sparkes, Steele, Symonds, D. Smith, Tempelaar, Twell, Wu)

**Cereals.** Following assessment of the culture responses of over 60 genotypes of wheat and barley, studies on somaclonal variation in wheat plants regenerated from cultured immature tissues have continued, with field assessments of $R_2$ and $R_3$ generations (at Nickerson RPB Ltd, with Dr J. T. Semple), and analysis of gliadin storage proteins of grains from $R_2$



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regenerants (with P. R. Shewry). The chromosome complement of somaclones showing variant gliadin patterns and field performance have been analysed. All the gliadin variants were cytologically normal, and are now undergoing molecular analysis. About 50 barley plants regenerated from culture were screened for seed proteins (hordeins) and cytology. One regenerant with chromosome breakage and a variant hordein pattern was identified. Following extensive testing of about 600 hexaploid wheat suspension culture lines, assessed for morphogenetic capacity and protoplast culture, a reliable bread wheat suspension culture system has been developed yielding protoplasts that divide and form callus. This system is being used to examine transient gene expression (with Dr S. Gleddie, PBI) and transformation of wheat by direct gene transfer using electroporation. Work is also in progress to attempt to transform multicellular embryogenic cereal tissues to obtain transformed wheat plants.

Joint experiments (with the University of Leeds) to examine cereal lectins in culture, during embryogenesis and embryo development are in progress. The aim is to identify markers for embryogenesis.

**Potato.** Work on regeneration of plants from potato protoplasts has continued. The aims include: increasing the range of useful cultivars that can be manipulated, understanding crucial early events in cell division and their effects on regenerated plants, and application of knowledge gained to genetic manipulation of potato (fusion, transformation). The efficiency of regeneration of plants from protoplasts of important potato cultivars (Maincrop: cv. Maris Piper, Désirée; First early: Maris Bard) has been considerably improved, with 64% of cv. Maris Bard regenerants being euploid (2n=4x=48). Work to regenerate plants from a range of dihaploid protoplasts (in collaboration with Scottish Crops Research Institute, Pentlandfield) and diploid wild species is in progress.

**Fusion.** In this work the transfer of larger blocks of genetic information than can be introduced by vectors is being studied. In particular, transfer of disease resistance traits between cultivars, or from wild species, is being examined. Analysis of early events after fusion of irradiated protoplasts of *N. sylvestris* ($Aec^R$) and potato has continued. Somatic hybrid plants have been successfully regenerated following fusion of protoplasts of cv. Maris Piper and *S. brevidens* (diploid wild species with potato leaf roll virus resistance). Hybrids are being characterized by isoenzyme analysis, specific DNA probes and chloroplast DNA restriction patterns. Hybrid cell lines obtained from electrofusion of 5MT and $Aec^R$ dihaploid potato protoplast lines have been identified and characterized successfully (with University of Groningen, The Netherlands).

**Agrobacterium.** Following the establishment in previous years of reproducible transformation procedures for potato and also for oilseed rape, research this year has focused mainly on further molecular and physiological analysis of transformed plants. Examination of stability of introduced characters has continued, and additional transformed lines have been isolated to examine variation. In potato, expression of foreign DNA appeared stable, but there was some indication that this was not so in oilseed rape. The use of short life-cycle plants of oilseed rape has facilitated the isolation of additional transformed plants.

## Disease resistance

Our joint programme (with Plant Pathology) on pathogenesis-related (PR) proteins has progressed rapidly over the last 18 months. The kinetics of synthesis and location of the major PR proteins with respect to TMV and local lesions is consistent with them being involved in, or closely associated with, restricting the spread of virus (see Plant Pathology



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report for further details). These proteins are also induced by spraying leaves with salicylic acid and we have studied the regulation of synthesis by isolating cDNA clones for these proteins. A further group of proteins of slower relative mobility have also been characterized and studied. (Area coordinator: Miflin; Antoniw, Buxton, Heyworth, Pierpoint, with White, Plant Pathology)

**Pathogenesis-related proteins**

***Location.*** The pathogenesis-related (PR) proteins of tobacco leaves responding hypersensitively to tobacco mosaic virus are thought to be mainly located in the intracellular spaces of the leaves: this is true of the well-known PR's-I and -II that migrate rapidly on electrophoresis, and also the slower migrating PR's -N, -O, -P, -Q and -R. They can be gently and almost quantitatively extracted from the leaves without disrupting any cells, following vacuum infiltration of water or buffer into the intracellular spaces. Some of the factors involved in maximizing the extraction of intracellular fluid and of concentrating the extracted fluids have been examined. The gentleness of the procedure makes it likely that the proteins are extracted with minimal modification and so are likely to retain any functional activity they possess. These extracts are now being tested for various biological properties including the ability to interfere with the translation and replication of TMV in different sytems.

Intracellular fluids have also been examined for virus-induced peroxidases, because peroxidase-catalysed lignification has often been implicated with the induced resistance to further infection that develops in infected plants. Induced isozymes of peroxidase can indeed be detected in the intracellular fluid of virus-infected leaves. But neither they nor the constitutive peroxidases can be identified as PR proteins; unlike PR proteins they behave as glycoproteins and are absorbed onto columns of immobilized concanavalin A.

It would be useful to study PR protein formation and export in protoplasts. However protoplasts are normally isolated and maintained in strong mannitol solutions and mannitol sometimes stimulates PR protein production. To test this effect, 6 mm diameter leaf discs were floated on mannitol solutions. Under these conditions they usually accumulated PR-Ia protein but this was suppressed when antibiotics were added to the mannitol, suggesting that contaminating microorganisms are responsible for mannitol-induced PR-Ia production. Increasing concentrations of mannitol progressively suppressed salicylic acid-induced PR-Ia production up to 98%. Total protein synthesis, determined by labelling with $^{35}S$ methionine, showed similar decline. However, a large proportion of PR-Ia produced by leaf discs entered the surrounding medium and this release did not appear to be affected differentially by increasing mannitol concentration.

Protoplasts were isolated from Xanthi-nc tobacco leaves and treated with salicylic acid. The amount of PR-Ia was measured by ELISA and found to increase from 120 to 2500 pg $10^{-6}$ protoplasts over 48 hr. About 90% of the PR-Ia was secreted into the culture medium.

**Isolation of cDNA clones**

PR-Ia was purified from TMV-infected Xanthi-nc tobacco and cleaved with cyanogen bromide to give one major and one minor peptide. The major peptide was sequenced and 20 residues identified. All possible coding sequences for this sequence were deduced and then a 17 base long oligonucleotide probe constructed and used to probe a cDNA library. This library was constructed in $\lambda$gt 10 from poly $(A)^+$-RNA isolated from Xanthi-nc tobacco leaves sprayed with salicylic acid. One clone of about 500 bp has been identified and sequenced. It contains the complete coding sequence for the protein. Using this clone we have demonstrated that the changes in the amount of PR-Ia and the other related PR-I proteins in response to TMV infection or spraying with salicylic acid, are due to large



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increases in the amount of mRNA present in leaves. (With Dr J. Taylor and Dr S. Pemble, Middlesex Hospital and Dr D. Pappin and Dr J. Findlay, University of Leeds)

### Cereal seed proteins

A reorganization of research areas within the Department has resulted in combining studies of the structures of seed proteins and their genes into a single area. The range of approaches varies from spectroscopic studies of protein conformation and dynamics to the fine structural analysis of seed protein genes. The main area of expansion is the study of wheat gluten proteins and their relationship to technological properties. This expansion is related to the increased production of wheat in the UK, and of gluten by industrial separation processes. It is anticipated that a surplus of gluten will soon result in its increased use in the food industry and elsewhere. Aspects of the work are described in more detail below. (Area coordinator: Shewry; Baker, Bunce, Burgess, Byers, Festenstein, J. Forde, Franklin, Halford, Henderson, Karp, Kreis, Lyons, Makin, Parmar, Pywell, S. Smith, Tatham)

**Identification of $\gamma$-type prolamins in barley and meadow grasses.** We have classified the prolamin storage proteins of barley, wheat and rye into three groups on the basis of their amino acid sequences and the chromosomal locations of their structural genes (see Shewry, Miflin & Kasarda, *Philosophical Transactions of the Royal Society* (1984) B304, 297–308). Of these one group, called the sulphur-rich (S-rich) prolamins, is the quantitatively major group in all three cereals, and the most diverse in structure. Detailed comparisons of amino acid sequences indicate that the $\gamma$-gliadins of wheat and the $M_r$ 40000 $\gamma$-secalins of rye represent the ancestral type of S-rich prolamin, whereas the other S-rich prolamins of these species and B hordein, apparently the only S-rich prolamin present in barley, are derived forms. We have now shown (in collaboration with Dr D. D. Kasarda, USDA, California, USA) that prolamins with $\gamma$-type N-terminal amino acid sequences are present in barley. Although these are only minor components in normal barley cultivars, they are the only S-rich prolamins present in the mutant line Riso 56, which has been shown to have a deletion of genes at the *Hor 2* locus encoding B hordeins. Further evidence that $\gamma$-type prolamins are an ancestral type comes from our demonstration that prolamin fractions from five species of meadow grasses (*Lolium perenne, Festuca rubra, F. arundinacea, Dactylis glomerata* and *Phleum pratense*) from two tribes (the Festuceae and the Agrostideae) also have $\gamma$-type N-terminal amino acid sequences.

**Characterization of cereal prolamins.** We have studied the structure and dynamics of whole gluten and gluten sub-fractions by NMR spectroscopy (in collabortion with Dr P. Belton, Food Research Institute, Norwich). No correlations between spectral characteristics and baking quality were observed when whole gluten fractions from a range of cultivars were studied using $^{13}C$ solid state NMR. This procedure did, however, show different populations of mobile and immobile carbon atoms in gliadin and glutenin fractions. Relaxation time experiments showed differences in the mobilities of protons between glutens from good and poor quality cultivars and in gluten sub-fractions. Whereas the gliadins are fairly rigid, the glutenins are more mobile.

C hordein of barley has also been studied in the solid state and in solution. It gives a remarkably simple $^{13}C$-NMR spectrum for a protein of over 50000 molecular weight, and most of the resonances can be assigned to the five predominant amino acids. The simplicity of the spectrum is consistent with the repetitive primary structure of the protein, and the presence of a regular secondary structure (*Rothamsted Report for 1984,* 154–155).

Further isolation of genomic clones for wheat gluten proteins has been achieved and sequence information is being obtained.



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**Content of sulphur in British wheat.** Most of the sulphur in wheat grain occurs in the protein amino acids cysteine and methionine, and the concentration of cysteine is one of several factors that can influence the breadmaking quality of wheat flour. The ratio of nitrogen to sulphur in the grain can be diagnostic of quality for bread, and values larger than 17:1, the suggested critical value, may indicate deficiency of sulphur. Sulphur inputs to agriculture have been decreasing with the increasing use of fertilizers containing no sulphur and diminishing deposition of sulphur from the atmosphere. It is against this background that the sulphur and nitrogen contents of wheat grain have been determined.

Samples of grain obtained by the Home-Grown Cereals Authority in its 1981 and 1982 surveys were analysed, giving sulphur contents between 0·13 and 0·22%, and nitrogen/sulphur ratios from 9 to 15. The sulphur contents of different wheat cultivars varied to a limited extent, with Copain (a Class I breadmaking cultivar) consistently containing the most sulphur. A high concentration of nitrogen rather than low sulphur accounted for most of the samples having high N/S ratios. High levels of total sulphur and sulphate sulphur occurred in all cultivars where atmospheric pollution from industry was expected. The sulphur contents were largest in the Midlands and North of England and smallest in NE Scotland, where sulphur deficiency is known to occur. The results illustrate the dependence of crops on sulphur derived from the atmosphere. (With McGrath and R. Webster, Soils and Plant Nutrition)

## Gene isolation and expression

Biochemical and physiological functions are dependent on the expression of defined genes. Functional differences between cells, tissues and organs exist because genes are differently expressed. Changes in metabolism within a given tissue are also achieved by controlling the expression of genes in relation to various stimuli—for example environmental signals. We are interested in a number of specific plant functions of critical importance in determining crop productivity and quality that are affected by the specific expression of defined genes. These include the storage of protein and carbohydrate in potato tubers and cereal seeds since the storage process is critical in determining the quantity and quality of the majority of harvested material from UK crops. Advances in recombinant DNA technology are also allowing us to investigate the control of a range of physiological processes in plant development and these are, and will continue to be, followed particularly with respect to potato. Finally, the assimilation of ammonia is a critical step in the pathway of nitrogen from the external environment into protein and provides a model example of how plant function may be altered by the expression of specific genes in response to an external event (in this particular case infection of legumes by effective rhizobia). Selected aspects of this programme are reported below. (Area coordinator: Miflin; Brooks, Burgess, Burrell, Buxton, J. Clark, B. G. Forde, J. Forde, Gebhardt, Gibson, Halford, Hammond, Henderson, Heyworth, Keddie, Kreis, Kruger, Leatham, Malpica, Marris, Oliver, Ooms, Pywell, Saarelainen, Schmutz, Tillson, Twell, Williamson)

**Developing cereal endosperms.** The genes encoding the barley seed storage proteins and $\beta$-amylase are expressed during the same developmental stage, while the chymotrypsin inhibitors (CI-I and CI-II) and other proteins are expressed about ten days later. These proteins, of which $\beta$-amylase and the chymotrypsin inhibitions are relatively rich in lysine, are also differentially expressed in different high-lysine barley mutants. We are, therefore, interested in the structure of these genes and in the mechanisms that regulate their expression. This year we have isolated several full length chymotrypsin inhibitor and $\beta$-amylase cDNA clones. Their nucleotide sequence has been determined and their amino acid sequence deduced. We have used the CI-II DNA probe to determine gene number in barley



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and wheat, and to estimate mRNA abundance in normal and high-lysine barley lines. Our results show that: (i) CI-II is encoded possibly by 4 to 6 genes/haploid genome; (ii) some barley varieties show polymorphism in the hybridizing restriction fragments, (iii) two different sub-families of CI mRNA are found in the endosperm, (iv) the rates of synthesis of chymotrypsin inhibitors in cultivars and mutant lines are related to the abundance of the mRNA, and (v) the putative signal sequence encoded by the CI-II mRNA contains an 'in frame' ochre stop codon. We are currently isolating and characterizing genomic clones of $\beta$-amylase and CI-I and II in order to study the *cis*- and *trans*-acting factors responsible for this coordinated and differential expression.

The complete nucleotide sequence of a 'B' hordein gene and its flanking regions has been determined. The cloned gene contains no introns and belongs to the 'B1' sub-family of 'B' hordeins. Comparison of the 5′ flanking sequences with those of related S-rich prolamin genes from wheat shows that several short sequences within 600 bp upstream of the translation initiation codon are strictly conserved. In particular a sequence at around −30 bp is conserved in similar locations in genes for 'B1' hordeins and the major classes of zein. Such a conserved sequence is unlikely to have occurred by chance and may have a function in the control of expression of the prolamin genes. To further the study of the control of expression of 'B' hordein genes, transcriptionally active nuclei have been isolated both from endosperm and leaf tissue of barley. Our preliminary results indicate that endosperm nuclei contain proteins that bind to the 5′-end of the 'B1' hordein gene. We are also identifying regions of the genes, in isolated chromatin from different organs, that are protected from nuclease (*Exo*III) digestion or chemical reaction or, conversely, are hypersensitive to DNAase. These techniques should identify regions of the genes that interact with proteins in the native state and are expected to be involved in the control of transcription. To attempt a functional analysis of 5′ regions, isolated DNA from barley containing a 'B1' hordein gene has been inserted into the *Eco*RI site of the T-DNA containing plasmids Bin6 and Bin19 (Bevan, *Nucleic Acids Research* (1984) **12,** 8711–8712). The construct was transferred into *A. tumefaciens* LBA4404, which contains the Ti plasmid pAL4404, to form a binary vector. Transformed tomato plants are currently being produced by a leaf disc transformation method.

**Potato tubers.** The major form of protein deposited in potato tubers is called patatin. In conjunction with Prof. W. Park of Texas A and M University we have isolated genomic clones for this protein. As a first step in understanding factors controlling their expression we have analysed the structure of the genes and are determining their sequence. From results so far it is clear that patatin is encoded for by a multigene family of which the individual members are relatively complex and contain large intervening sequences.

Quality in potato tubers is also affected by the content of soluble sugars and we have initiated a programme to isolate and study enzymes that are thought to regulate sucrose accumulation. The first enzyme chosen is phosphofructokinase and this has been purified to virtual homogeneity—something that has not been previously achieved for the enzyme from any plant source.

**Control of expression of glutamine synthetase.** Glutamine synthetase (GS) in plants occurs in a number of different isoforms that are associated with different organs and cell compartments. The complete amino acid sequences of two subunits of the root form of GS in *Phaseolus vulgaris* have been obtained by nucleotide sequencing of two full-length GS cDNA clones. Comparison with mammalian and cyanobacterial GS sequences indicates that they have all evolved from the same ancestral gene. A number of genomic clones have been isolated and mapped by restriction enzyme analysis (in collaboration with Dr J. V. Cullimore, University of Warwick). One of the cloned GS genes is specifically expressed in the



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root nodule, while another appears to be expressed throughout the plant. We are investigating the molecular basis of the differential regulation of this family of closely related genes.

### Glasshouse services

This year the development of the central glasshouse area for the Molecular Sciences and Agronomy and Crop Physiology Divisions was completed, providing the glasshouse services section with 170 $m^2$ of new automated glass and 300 $m^2$ of new refurbished header/service areas. With the occupation of two glasshouses formerly used by Soil Microbiology, glasshouse services now manage eight glasshouses (16 compartments) with a floor area of 910 $m^2$. The computer logging and monitoring system provided in the new glasshouse is being extended to include all of the service glasshouses.

During the year the glasshouse services section provided about 16000 pots and trays for the two divisions and has continued to support and develop work on the growth of cereals under defined nutrient regimes. (Services coordinator: Franklin; Hackett, with Plumb and Ward, Physiology and Environmental Physics)

### Staff and visitors

**Outside support and collaboration.** The Department gratefully acknowledges the financial support for personnel and materials that have been provided by the Agricultural Genetics Company, the Home-Grown Cereals Authority, Shell Research Ltd, the Potato Marketing Board, Directorates DG XII (Education and Science) and DG VI (Agriculture) of the EEC, Sigma Chemical Company, NATO, Limagrain, ICI Plant Protection, the International Potato Centre, The Gatsby Foundation, British American Tobacco Co Ltd and De Danske Sukkerfabrikker. We have also shared SERC/CASE awards with the Universities of Durham and Warwick.

**Visitors.** During the year the Department was pleased to welcome for extended visits: Miss Denise Gerentes (France), Dr N. Kruger (Toronto), Dr A. Marquez (Spain), Miss Eevi Rintamaki (Finland), Dr A. Shah (FRG), Mr D. Smith (University College, London), Mr Qinsheng Wu (China)

**Visits abroad.** Members of the Department attended the following conferences: Plant Flavonoid Conference, Buffalo, USA (W. S. Pierpoint); Gordon Research Conference on Plant Cell and Tissue Culture, Plymouth, USA (A. Karp, S. Maddock); EEC Symposium on Somaclonal Variation and Crop Improvement, Gembloux, Belgium (A. Karp, S. Maddock); Eucarpia meeting on Genetic Manipulation and Plant Breeding, Berlin, W. Germany (M. de Both, N. Fish, D. Foulger, A. Karp, G. Ooms); EMBO course on Transfer and Expression of Genes in Higher Plants, Cologne, FRG (S. Maddock); EMBO workshop on DNA Infectious Agents, Rorschach, Switzerland (M. G. K. Jones); 1st International Congress of Plant Molecular Biology, Savannah, USA (L. de Bry, M. Kreis, S. Maddock, B. J. Miflin, G. Ooms, R. Saarelainen); UNEP course, Shanghai, China (R. Wallsgrove); 13th International Congress of Biochemistry, Amsterdam, The Netherlands (B. Forde, M. Kreis, B. J. Miflin, M. Williamson); Conference on Biological Control of Photosynthesis, St Truiden, Belgium (S. Gutteridge, A. Kendall, M. Parry); ASPP Meeting, Rhode Island, USA (R. Wallsgrove); UCLA Symposium on Protein Structure, Folding and Design, Denver, USA (P. R. Shewry); 2nd International Symposium on Biochemical Approaches to Identification of Cultivars and Evaluation of Their Properties, Braunschweig, FRG (P. R. Shewry); EEC Meeting on Protein Evaluation in Cereals and Legumes, Thessaloniki, Greece (B. J. Miflin, P. R. Shewry)



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Visits abroad were made to the following institutions to further collaboration or for exchange of information: USDA Laboratories, Albany, USA (J. Forde, P. R. Shewry); ARCO Plant Cell Research Institute, Dublin, USA (B. G. Forde, P. R. Shewry); University of California, Berkeley, USA (B. G. Forde); Universities of Leiden, Wageningen, and Groningen, The Netherlands and Wisconsin and Texas A & M University, USA (G.Ooms); Agrigenetics Company, Madison, USA (G. Ooms, P. R. Shewry); Pioneer Hybrid, Johnston, USA (S. E. Maddock); University of Groningen (M. G. K. Jones); Biosem, Clermont-Ferrand, France (M. de Both); INRA, Nantes, France (A. Tatham); American Cyanamid Agricultural Research Division, Princeton, USA (R. M. Wallsgrove); University of Leiden, The Netherlands (B. G. Forde, M. Kreis, C. Marris, B. J. Miflin, M. Williamson)

**Staff.** G. Festenstein retired after 27 years, B. J. Miflin left the Department after 12 years to take up an appointment of Head of R & D of Ciba-Geigy Seeds Sub Division in Basel. During the year N. Bunce, Nikki Darrell, J. M. Hill, Val Jones, Sheila Maddock, P. B. Norbury and A. Parton also left.

Newcomers to the Department were: Amarjit Bains (Lanchester Polytechnic), Pat Brooks, Melody Clark (University of London), C. Eady (University of Sheffield), Barbara Hackett, J. Hammond (GCRI), Janina Jones, M. Keddie (University of Edinburgh), Catherine Kettleborough (Brunel University), Christine Leatham (University of Leeds), Alison Lyons (University of Durham), A. Parton (University of Lancaster), A. Phillips (Cambridge University), R. Potter (University of Leeds), J. Relton (University of Leeds), Susan Steele, and Penny Tillson (Open University). S. Bright is spending a year at ARCO Plant Cell Research Institute, Dublin, California and J. Antoniw a year at the University of Leiden.

## PUBLICATIONS

### Books

BRIGHT, S. W. J. & JONES, M. G. K. (Eds) (1985) *Cereal tissue and cell culture.* Dordrecht: Nijhoff/Junk.

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