ROTHAMSTED RESEARCH

Report for 1979 - Part 1

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Biochemistry Department

B. J. Miflin

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BIOCHEMISTRY DEPARTMENT

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Introduction

The Department has expanded during the year as new people involved in the genetic manipulation programme have been appointed. The overall aim of this programme is to develop techniques which will enable genetic information to be introduced into crop plants in ways which cannot be achieved by conventional plant breeding. One area in which we are involved is the isolation of the genetic information specifying the storage proteins of cereals, particularly barley. Once we have achieved this the aim is to modify the DNA to specify a more desirable protein (from the standpoint of animal nutrition) and reintroduce it into the plant. We have also started a new programme to establish techniques which will allow us to regenerate whole crop plants from single protoplasts. Two particular benefits envisaged from this work are the ability to introduce novel DNA into protoplasts and also to be able to fuse protoplasts from species that do not normally cross. A further advantage may accrue from the use of regenerating protoplasts in our mutant selection studies.

The Department's interest in host/pathogen relationships has continued. This work is done in close collaboration with members of the Plant Pathology Department and in some cases is reported under that Department. In particular the study of pathogenesis-related proteins has revealed many interesting new facets.

Our studies on intermediary nitrogen metabolism have moved away from the primary mechanism of ammonia assimilation, now that the pathway that we proposed is no longer a matter of controversy and have been more concerned with the secondary re-

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assimilation of ammonia and the control of amino acid biosynthesis. Ammonia re-assimilation is quantitatively much more important than primary assimilation; that occurring during photorespiration alone exceeds the primary rate. Further reassimilation occurs during the utilisation of transport compounds such as asparagine, arginine, allantoin and allantoic acid for protein synthesis (e.g. in the storage organs of developing seeds). The control of the synthesis of lysine, methionine and threonine appears more subtle and complicated the more it is studied. We hope that our combined approach using biochemical studies allied to the selection of presumptive regulatory mutants will enable us to understand the various mechanisms that operate.

Host/plant pathogen relationships

Virus studies. The aims of this work are to identify the nature and function of novel proteins that are formed in plant leaves in which the spread of invading virus is restricted; and to describe some of the surface features of potato virus X (PVX) that affect its behaviour in senescing infected leaves.

Properties of different strains of potato virus X (PVX). Cultivated potatoes contain two major genes which induce a local hypersensitive reaction to infection with PVX, and hence confer resistance against this virus. Common strains of the virus belong to one of three groups depending on whether they provoke such a response from either one of these genes or from both. Fortunately, strains which react with neither gene, and against which potatoes have no hypersensitive resistance, are rare, and only two are known to us. One of these, X^4, has already been described, and the other, X_{HB}, was isolated from Bolivian sources by Dr. R. A. C. Jones. We have examined both these strains for properties, especially susceptibility to proteolytic enzymes and reaction with generated quinones, that may affect their survival *in vivo*, and which allow us to compare the structure of their protein subunits with those of common strains.

We have confirmed that X^4 contains only one type of subunit, which is slightly larger than that of a number of common strains. Unlike the protein of these strains, it is resistant to brief digestion with trypsin, and binds half a molecule of chlorogenoquinone without being inactivated. Its subunits are cross-linked by di-imidates as are those of the common strain X^N. X^4 is, however, unstable to serial inoculation in Xanthi tobacco, and progressively changes to forms which more closely resemble X^N although they may retain resistance to trypsin: it is not yet known if these forms have acquired the ability to induce a hypersensitive response in potatoes. Strain X_{HB}, which produces few if any symptoms on Xanthi tobacco at normal glass-house temperatures, may also change gradually on serial subculture (Dr. R. A. C. Jones). However the size of its protein subunits and their reaction with chlorogenoquinone resemble those of X^N, and only its trypsin-resistance resembles that of X^4. Although this suggests that X^4 and X^N have some structural features in common, it provides no explanation for their rarity in nature. (Pierpoint and Strowman, with Carpenter, Plant Pathology Department)

Nomenclature of virus-induced plant proteins associated with resistance. Several arbitrary and incompatible systems of nomenclature exist in the literature for the novel proteins that are formed in leaves following the formation of localised necrotic lesions by viruses. We have, therefore, with others, proposed a new and more comprehensive system in which they are referred to as pathogenesis-related proteins or PR-proteins. For each plant species or cultivar, they are classified on the basis of their mobility during electrophoresis in polyacrylamide gels (PAGE), and grouped together into families with similar molecular weights but different charges.

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The estimation and synthesis of PR-proteins in TMV-infected plants. Attempts to quantify PR-proteins in extracts of tobacco (var. Xanthi-nc) leaves, by spectroscopically scanning stained electrophoresis gels are complicated by the presence of many proteins in the extracts, especially one which migrates similarly to PR-Ia (formerly b_1). Much of the interference can be removed by pre-incubating the extract with proteolytic enzymes, which have little effect on PR-proteins, or by using gels made from 15% acrylamide. Although the resulting procedure is only semiquantitative, it is adequate for following the synthesis of PR-proteins both in intact plants and in detached leaves.

PR-proteins, especially PR-Ia, are detectable in TMV-inoculated leaves of Xanthi tobacco 3 days after inoculation, and reach a level of 9–10 $\mu g\ g^{-1}$ fresh weight after 7 days, when they comprise about a tenth of the protein that can be extracted at pH 2·8. They appear in adjacent, uninoculated leaves 7 days after inoculation, and accumulate to a smaller extent. They are also formed in uninoculated leaves of plants from which the inoculated leaves have been removed not less than 3 days after inoculation. It may be, therefore, that during these 3–4 days some chemical messenger is produced in inoculated leaves and transported to adjacent leaves where it initiates the synthesis of PR-proteins. Preliminary attempts have been made to extract such a messenger and detect it by its ability to initiate synthesis in detached leaves. Protein-free extracts of infected leaves have this ability, but so do extracts of healthy leaves. These extracts are complex and they damage treated leaves. It is not yet known if they contain a specific component which induces PR-proteins or if it is the unspecific stress which they impose that initiates the synthesis. (Antoniw, Pierpoint, Robinson and Strowman)

Comparison of PR-proteins in different tobacco cultivars. Infection of leaves of five cultivars of *Nicotiana tabacum* with necrotic lesion-forming viruses induced resistance to further infection by these viruses and at least four PR-proteins in each cultivar. The electrophoretic behaviour of these proteins showed that the four PR-proteins in Xanthi-nc were essentially electrophoretically identical with those in the Xanthi, Samsun and Samsun NN cultivars. White Burley also contains proteins identical with the three PR-1 proteins in the other cultivars as well as an additional PR-1 type protein.

Previous work has shown that treatment of Xanthi-nc with polyacrylic acid (*Rothamsted Report for 1974*, Part 1, 117) and aspirin (*Rothamsted Report for 1978*, Part 1, 207) also induces resistance to virus infection and the appearance of three PR-proteins. Injection of these chemicals into leaves of the other four cultivars showed that polyacrylic acid induces resistance and PR-proteins in only Xanthi-nc and to a lesser extent in Xanthi, whereas aspirin induces resistance and PR-proteins in all five cultivars. (Antoniw, with White, Plant Pathology Department)

Fungi of the* Gaeumannomyces–Phialophora *complex. Cell-wall degrading enzymes of several new isolates were studied to help with their identification. The results obtained with two of these isolates are discussed in the report of the Plant Pathology Department (p. 171).

Further observations on the growth of a range of fungus isolates on agar containing roots or leaves of oat seedlings has shown marked differences between oat cultivars. However, the results confirm that the test is useful in helping to distinguish different types of fungi in the complex. (Holden and Dodds)

Intermediary nitrogen metabolism

Ammonia assimilation and reassimilation. The assimilation of ammonia produced during nitrogen fixation in nodules of *Phaseolus* takes place via the action of the enzyme gluta-

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mine synthetase. The activities of this enzyme and of two enzymes, glutamate synthase and glutamate dehydrogenase, potentially capable of producing glutamate in the nodule during the course of its development have been followed. The results show that the specific activities of glutamine synthetase and glutamate synthase are many times those in uninoculated root tissue and that the levels of the enzymes increase before nitrogenase activity rather than after it. The enzymes, therefore, are not formed in response to the presence of ammonia from nitrogen fixation but they may ensure that conditions in the nodule (i.e. very low ammonia levels) are conducive for nitrogenase synthesis. The localisation of these enzymes within the root nodule has been studied further. Glutamine synthetase is predominantly in the cytoplasmic fraction of the plant part of the nodule with about 10% of the total activity being in the plastids. Most of the glutamate dehydrogenase was located in the bacteroid, that present in the plant fraction (*c.* 15% of the total) was exclusively located in the mitochondria. Glutamate synthase was largely in the plastids.

We have previously reported (see last year's annual report) that glutamine synthetase in leaves is present in the cytoplasm and chloroplasts but not the mitochondria. However other workers (Jackson *et al. Biochemical Society Transactions* (1979) **7,** 1122–1124) have suggested that mitochondria contain the enzyme and that it is capable of reassimilating the ammonia released in the conversion of glycine to serine during photorespiration. Using a number of different techniques for the isolation and separation of cell organelles we (with Keys, Bird and Cornelius, Botany Department) were unable to demonstrate either the presence of glutamine synthetase or find evidence of ammonia assimilation in mitochondria. Only when mitochondrial electron transport is inhibited and relatively high concentrations of ammonia added could we observe the slightest suggestion that glutamate dehydrogenase might be catalysing the synthesis of glutamate. The results were all consistent with the action of the photorespiratory cycle as previously published (Keys *et al.*, *Nature, Lond.* (1978) **275,** 741–743).

Amino acid biosynthesis. The synthesis of lysine, methionine and threonine has been studied in a number of tissues. Feeding ^{14}C-aspartate to isolated, developing pea cotyledons showed that they could form lysine, threonine and isoleucine. The synthesis of methionine was not clearly demonstrated, probably due to problems in reproducibly isolating the compound. Lysine, threonine and isoleucine were each able to prevent their own synthesis when added at 8 mM. Methionine synthesis was demonstrated in young barley roots and was found to be regulated by exogenously added methionine and lysine plus threonine.

Studies have continued on the regulatory mechanisms that operate at the level of feedback control of two of the enzymes (aspartate kinase and homoserine dehydrogenase) of the pathway. Different forms of these enzymes appear to exist in most of the tissues so far studied and the proportions of the different forms change during the development of the tissues. Aspartate kinase isolated from young seeds of pea and field bean shortly after flowering were sensitive to 10 mM lysine. The sensitivity to lysine remained constant throughout maturation until the seed began to dry out when there was a drop in both lysine sensitivity and total activity. Homoserine dehydrogenase isolated from immature pea seeds is sensitive to both threonine and cysteine; as the seeds mature the total activity becomes less sensitive to threonine and more sensitive to cysteine. With pea and barley leaves the threonine-sensitive activity could be separated from the cysteine-sensitive activity. Chloroplasts contain a threonine-sensitive enzyme which is inhibited only by high concentrations of cysteine whereas the cytoplasm contains a homoserine dehydrogenase insensitive to threonine, but greatly inhibited by small amounts of cysteine. The two forms of the enzyme can also be separated by chromatography on Blue-Sepharose

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or, to a lesser extent, on Sephadex G-200. In barley leaves the sensitivity of the chloroplast enzyme to threonine decreases as the leaves mature.

Inhibitor studies. We have been investigating the synthesis of inhibitors of potential use in determining the relative importance of different enzymes in pathways of nitrogen metabolism. We have been particularly interested in finding inhibitors of glutamine synthetase, glutamate dehydrogenase, glutamate synthase and saccharopine dehydrogenase. Although a number of compounds have been synthesised so far none have been exceptionally effective.

Selection of biochemical mutants. We have continued to screen for biochemical mutants of barley resistant to lysine plus threonine (LT) or aminoethylcysteine (AEC). Mutants are defined as those plants which grow well under the selective conditions and also transmit this character to their offspring. We have isolated six LT-resistant mutants from $1{\cdot}6 \times 10^4$ M_2 embryos; in five the resistance is dominant and in the other probably recessive. Of three AEC-resistant mutants one at least is determined by a recessive gene (see last year's report, p. 29). The LT-resistant mutant R2501 upon selfing gave rise to resistant and sensitive progeny in the ratio of 1·5:1. Sensitive progeny upon selfing gave rise only to sensitive plants, whereas offspring of resistant plants all segregated for resistance as before, except that this was closer to the 2:1 ratio expected of a single dominant gene lethal in the homozygous condition. Heterozygous resistant plants grown on normal medium contained increased amounts of free methionine (×2), threonine (×4) and lysine (×1·4) compared with either sensitive progeny or the parent variety. Another LT-mutant, R3202, has an increased content of methionine (×2) but not of lysine or threonine. (Awonaike, Bright, Gill, Hill, Kueh, Lea, Leason, Mayne, Miflin, Norbury, Parkin, Petzing, Rognes, Sainis, Smith, Thomson and Wallsgrove)

Storage proteins in cereals

Protein extraction and characterisation. The storage protein fraction (hordein) of barley was further characterised. Hordein consists of two groups of polypeptides (termed *B* and *C*). Whereas the *C* polypeptides are readily soluble in, and extracted by, 60% ethanol at room temperature, the *B* group is more soluble in, and more efficiently extracted by 50% propan-1-ol or 45% propan-2-ol at elevated temperatures in the presence of 2-mercaptoethanol. However, the most efficient extraction conditions also extract some non-hordein polypeptides resulting in an increased lysine content of the hordein fraction. Amino acid analysis of the purified hordein fraction shows that *C* hordein contains more glutamine, proline and phenylalanine but less lysine and S-amino acids than *B* hordein. Equilibrium sedimentation analyses on the *B* and *C* groups separately showed that the preparations were reasonably monodisperse with molecular weights of approximately 32 000 and 52 000 daltons respectively. These values are considerably lower than those previously determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The hordein fractions have also been characterised by their immunological properties. Various solvents have been tried for dissolving hordein for immunodiffusion tests; besides 1% SDS hordein will also dissolve (at 1 mg ml^{-1}) in 1% deoxycholate, 1% Triton-X100 or 6M-urea. Extensive stirring of hordein with either distilled water or phosphate-buffered saline will dissolve sufficient hordein for diffusion tests although it is not thought that all polypeptides are equally soluble. Immunodiffusion tests show that antiserum raised to *C* hordein polypeptides cross-reacts with *B* polypeptides showing that the two groups have antigenic determinants in common.

The glutelins of maize and barley were also studied. After removal of salt-soluble

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proteins and of prolamins by using dithiothreitol or mercaptoethanol in alcohol, the glutelins were reduced, alkylated, and extracted by several systems. The proteins were examined by SDS-PAGE and by isoelectric focusing. Urea made the glutelins soluble, but also solubilised starch, which interfered with SDS-PAGE separations. SDS and other detergents were also tested for solubilisation of glutelins. One maize glutelin protein was differentially extracted by 40% urea. Barley glutelins often showed contamination by hordein. The use of dithiothreitol rather than mercaptoethanol in the alkylation procedure produced sharper bands in SDS-PAGE. Glutelins produced SDS-PAGE and isoelectric focusing patterns with many bands, with little evidence that any could be classed as a major storage protein.

Studies on the gliadin (storage protein) fractions extracted from milled wheat grain by different solvent systems and using different procedures (see last year's report) have continued. After extraction gliadin fractions were dialysed against water before freeze-drying. All those extracted from undefatted wheatmeal, but none from defatted material, formed a precipitate during dialysis which could be separated by centrifugation and freeze-dried separately. Both fractions were analysed by SDS-PAGE, and the supernatant found to contain a disproportionate amount of the high molecular weight polypeptides that had been extracted. When the results using a range of solvents were analysed it was found that defatting led to better extraction of gliadin in a state more suitable for further analysis; however, it decreased the extractability of the salt-soluble proteins. Of the solvents tested 70% ethanol and 55% propan-2-ol behaved similarly and were least effective in extracting nitrogen, including the complete range of polypeptides, unless 2-mercaptoethanol (2%) was also present at room temperature or above. In contrast 50% propan-1-ol extracted much more protein containing a representative selection of polypeptides even at room temperature without the addition of other compounds.

Genetic analysis of hordeins. The *C* and *B* polypeptides have been shown previously to be controlled by two structural loci termed *Hor-1* and *Hor-2* on chromosome 5. In conjunction with Mr. R. Pickering, Mr. I. T. Jones (Welsh Plant Breeding Station) and Dr. R. A. Finch (Plant Breeding Institute (PBI)) we have further mapped these loci in relation to other genes on chromosome 5. Attempts were also made to see if the loci could be further resolved. Two-dimensional and high-resolution one-dimensional gel electrophoretic analyses of seeds of F_2 and doubled haploid progenies of four intervarietal crosses gave no evidence of recombination within the loci. Based on the numbers analysed it is suggested that the maximum map distance covering one locus is 0·01 centiMorgans (cM). This is considerably more than required to encode separately each of the polypeptides governed by the locus. Thus the results were not inconsistent with previous proposals that these are complex loci. Genetic analysis of the progenies showed that *Hor-1* is 0·16 cM from *Hor-2* and the mildew resistant locus *M1a* lies between them 0·062 cM from *Hor-1* and 0·082 cM from *Hor-2*. Further results suggested that *Hor-1* is near to a yellow rust resistance gene *Yr4* and thus closest to the centromere. Results from Risø (J. Jensen, H. Doll and colleagues) also support this order and show that a second mildew resistant gene *M1k* is close to *Hor-1*. The relevance of these results to the distribution of hordein polypeptide patterns prevalent in European barleys has been established. When 164 varieties were analysed, 139 spring varieties could be divided into 26 different groups depending on their *B* and *C* patterns and the 24 winter varieties into nine groups; altogether 32 different groups were recognised. When the origins of these patterns were studied it was found that several patterns had been introduced from exotic varieties used as sources of mildew resistance. Thus the line Arabische was introduced as a source of resistance based upon the presence of the *M1as* gene (an allele of *M1a*) and of the 15 varieties analysed that have the *M1as* gene derived from Arabische, 12 also have

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the same *Hor-1* and *Hor-2* alleles (*C* and *B* hordein patterns) as Arabische, two have the *Hor-1* allele solely and only one variety has a completely different hordein polypeptide pattern. The usefulness of polypeptide analysis on single seeds was shown, two mistakes in assigning the parentage of the varieties being uncovered. In future it may prove of even greater value in following the presence of mildew resistant genes during crossing programmes.

Nucleic acid studies. We have reported previously that membrane bound polysomes, isolated from developing barley endosperms, support the *in vitro* synthesis of proteins, in a wheat germ system, that are similar to authentic hordeins. The messenger RNA (mRNA) associated with the polysomes was isolated and likewise shown to support *in vitro* protein synthesis. This mRNA fraction is polyadenylated (to about 6% of its total length) and contains molecules between 0·55 and 2·55 kilobases in length. The products of *in vitro* protein synthesis resembled hordeins being predominantly soluble in 55% propan-2-ol, and containing a small proportion of lysine compared with leucine. They had similar, but not identical, electrophoretic properties. The differences are probably due to the presence of a leader sequence on the products of mRNA translation which is removed *in vivo* during the passage of the proteins through the endoplasmic reticulum in the formation of the protein bodies. Further studies on the processing of the polypeptides are in progress. The mRNA fraction has been used to make DNA copies, via reverse transcription, and subsequently double stranded DNA, by R. Thompson at the PBI. Unfortunately, however, attempts to clone the double stranded DNA were unsuccessful. Recently new attempts have been made to produce cloned DNA copies of hordein mRNA, thanks to the hospitality of Dr. J. Bishop of Edinburgh University, and the first experiment has led to the production of some small clones. As yet no confirmatory evidence has been produced that the cloned material is definitely related to the hordein mRNA but the work is continuing. In the absence of cloned DNA we have been developing techniques for mRNA purification, DNA:RNA hybridisation and gel electrophoresis of DNA and RNA. These have generally been established successfully and a battery of techniques is available to further develop the programme to isolate the structural genes for hordein.

Technological aspects of cereal proteins. The storage proteins of wheat are considered to play an important part in determining the quality of wheat flour for baking. In contrast, the proteins of barley are generally detrimental in malting. We have, with the support of the Home-Grown Cereals Authority, been trying to relate studies of the biochemistry of these proteins to these technological aspects.

Previous studies by others have suggested that baking quality is related to the amount of aggregated protein in the flour. However, investigations have been handicapped by the failure to completely extract such material and its nature has not been defined clearly. The first step was to develop a solvent system able to solubilise, in an unreduced state, a large proportion of wheat flour protein. Many systems were tested and 0·01M-acetic acid–6M–urea-2% cetyltrimethylammonium bromide was finally adopted. Under mild conditions this solvent extracted about 98% of the total gluten nitrogen, which is in marked contrast to the 60–70% recovery often considered acceptable. When extracted material was examined by gel permeation chromatography on controlled-pore glass, it eluted as two well-defined peaks, the first of which was excluded aggregated material. Upon reduction and subsequent SDS-PAGE this peak was shown to contain mainly high molecular weight polypeptides. The aggregated peak was not obtained when the extract was reduced prior to chromatography showing that it was stabilised either directly or indirectly by disulphide bonds. The amount of aggregated material was found to be

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correlated with baking quality in a wide range of wheat varieties. In poor baking varieties the ratio of excluded to non-excluded material was about 0·2:1 while for a variety of high baking quality it was around 0·4:1. However the ratio of total high molecular weight polypeptides to those of lower molecular weight was constant for all varieties, regardless of baking quality.

Workers at the Brewing Research Foundation proposed that malting quality is related to properties of the hordein fraction and particularly its polypeptide pattern. We have developed procedures for extracting hordeins from germinating and malted grain and their subsequent analysis by SDS-PAGE. The patterns show little change during the early stage of this process and varieties can still be identified in malted grain. When a large number of varieties are considered we find that some groups classified by their polypeptide pattern contain varieties of similar malting quality but others contain a wide range of malting types and thus there is no easily discernible correlation. Further studies are planned on the relationship of other properties of hordein to individual malting characteristics.

Work has also continued on the S content of the seed since this may be of major importance in the relationship of the storage proteins to both malting quality barley and baking quality wheat. The aim is to define more carefully the distribution of S amino acids (particularly cysteine) within the different protein fractions and to establish the role of intermolecular disulphide bonds in forming protein aggregates. (Bahramian, Burgess, Byers, Faulks, Festenstein, Field, Forde, Hill, Kirkman, Kreis, Matthews, Miflin, Parmar, Rahman, Ray, Shewry, Smith and Wilson)

Protoplast, cell and tissue cultures

The long-term goal of this project is to introduce new genetic information into crop plants by manipulation of single cells, protoplasts and microspores *in vitro* in such a way that they can both take up and incorporate new genetic information and also regenerate into whole plants. Success would provide a system for use in plant biochemistry and physiology and possibly a means of rapidly enlarging the pool of genetic variability within important plant species. Currently, cultures are being established from barley, wheat, potato, sugar-beet and rape to determine their suitability as sources of totipotent single cells and protoplasts for genetic manipulation. (Bright, Hemsley, Shah and Thomas)

Staff and Visitors

Outside support. The Department gratefully acknowledges the financial support for personnel and materials that have been provided by various organisations including the Home-Grown Cereals Authority, Ciba-Geigy Ltd., Shell Research Ltd. with the SRC, NATO and the EEC. We also thank all those people who have cooperated with us both inside the Station and in many organisations outside.

Visitors. Besides even more day visitors than last year the Department was pleased to welcome Dr. S. E. Rognes from the University of Oslo for a stay of 10 months and Mrs. Jayashree Sainis from the Bhabha Atomic Research Centre, Bombay, Mr. F. C. Martin from the University of Nancy, and Dr. E. De Gryse from the Vrije Universiteit Brussels, for shorter periods.

Visits abroad. Members of the department attended many conferences abroad chiefly by invitation. S. W. J. Bright and B. J. Miflin visited the Friedrich Miescher Institut Basel where they gave seminars. E. Thomas and S. W. J. Bright attended an international

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workshop in Pavia, Italy on Plant Cell Cultures Results and Perspectives, where S. W. J. Bright gave a paper. A. J. Faulks, M. Kreis and B. J. Miflin attended an EEC workshop on the Biochemistry of Cereal Storage Proteins in Risø, Denmark. A. J. Faulks and B. J. Miflin attended and gave a paper at the Eucarpia conference on 'Cereal Variety Identification' in Helsingor, Denmark. B. J. Miflin and R. M. Wallsgrove attended and presented a paper at an international conference on Glutamine Metabolism at the University of Mexico. B. J. Miflin lectured at the Western Regional Laboratory, Berkeley, California, the University of Oslo, Norway and the Vrije Universiteit Brussels. P. J. Lea attended and gave a paper at a conference on 'The Origin of Chloroplasts' at the National Institute of Health, Maryland, USA and also lectured at and/or visited several universities in Canada and the Carlsberg Research Laboratories, Denmark. E. Thomas participated in the Gordon Conference on 'Plant Cell and Tissue Culture', Andover, USA, the 'Plant Breeding Symposium—2', Ames, USA, the International Protoplast Symposium, Szeged, Hungary where he presented a paper, and also visited and/or lectured at several laboratories in the USA.

Longer visits were made by J. Hill to research institutes and universities in Australia and J. F. Antoniw who spent one month on an EMBO fellowship at the University of Wageningen, The Netherlands. P. R. Shewry has a year's leave of absence to take up a NATO Fellowship at the USDA/SEA Western Regional Laboratory, USA and R. M. Wallsgrove is working for a year with Professor M. Mazelis at the University of California, Davis, USA.

Staff. During the year the members of Department were especially successful in passing examinations. It is a particular pleasure to record that Shirley Burgess and P. B. Norbury were awarded Bachelors degrees and R. M. Wallsgrove a Doctorate since these were gained by part-time study. Two of the full-time students in the Department K. O. Awonaike and Deborah Thomson also successfully completed their Ph.D. courses. During the year Stephanie Petzing and Gillian Watson resigned and were replaced by J. Franklin and Susan Wilson. Anne Hemsley and Margaret Ashby also resigned. Other appointments made during the year were M. B. Bahramian from Imperial College, E. Thomas who joined us from the Friedrich Miescher Institut, Basel, B. G. Forde from Edinburgh University, M. Kreis previously of the Risø National Laboratory, Denmark, V. Shah and Anne Hemsley. Jane Pearton became Jane Ray on marriage.

Publications

Theses

1 Awonaike, K. O. (1979) Studies on the Development and Properties of Ammonia Assimilating Enzymes in *Phaseolus vulgaris* L. Root Nodules. Ph.D. Thesis, University of London.

2 Thomson, D. M. (1979) A Study of Lysine Metabolism in *Pyricularia oryzae*. Ph.D. Thesis, University of London.

3 Wallsgrove, R. M. (1979) Nitrogen Assimilation in Legume Leaves. Ph.D. Thesis, University of London.

General Papers

4 Bright, S. W. J., Lea, P. J. & Miflin, B. J. (1980) The regulation of methionine biosynthesis and metabolism in plants and bacteria. *Ciba Foundation Symposium No. 72 Sulphur in Biology*. Ed. K. Elliott & J. Whelan Amsterdam: Excerpta Medica, pp. 101–114.

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5 BRIGHT, S. W. J., NORBURY, P. B. & MIFLIN, B. J. (1980) Isolation and characterization of barley mutants resistant to aminoethylcysteine and lysine plus threonine. *Proceedof IUBS/CNR Workshop*. In: *Plant cell cultures: results and perspectives*. Ed. F. Sala, *et al.* Amsterdam: North Holland Biomedical Press, pp. 179–182.

6 FOWDEN, L. (1979) From fertiliser nitrogen to grain protein: constraints and opportunities. In: *Scottish Plant Breeding Station Annual Report, 1978–79*, pp. 159–168.

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