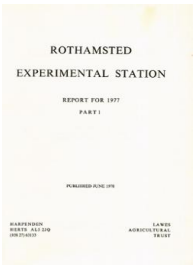
ROTHAMSTED RESEARCH

Report for 1977 - Part 1

Full Table of Content



Biochemistry Department

B. J. Miflin

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BIOCHEMISTRY DEPARTMENT

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Introduction

Little change has occurred in the major direction of the research work in the department which continues to be concerned with the biochemistry of host/plant pathogen relationships and of nitrogen metabolism of crop plants.

Host/plant pathogen relationships

Modified forms of potato virus X (PVX). We have previously described and characterised two forms of PVX, PVX-Q_1 and PVX-Q_2, which are produced by reaction with quinones formed during the enzymic oxidation of leaf phenols *in vitro*. These studies have suggested ways in which PVX is modified in ageing and senescing leaves and in which plant proteins in general are modified during extraction from phenol-rich tissue. They have also given insight on the surface structure of PVX and differences that exist between strains of the virus.

Natural modifications. A modified but intact form of PVX, PVX-M, was previously isolated from senesced infective leaves of *Nicotiana tabacum*, in quantities corresponding to about one-twentieth of the concentration of PVX in presenescent leaves. It is uninfective, but has some similarities to PVX-Q_1 and on saponification gives a fragment that reacts with periodate-thiobarbituric acid (PBTA). However, it is not certain that PVX-M is formed as a consequence of the generation of *o*-quinones by leaf *o*-diphenol oxidases. Although a similar material is detected in extracts made from green or yellowing leaves in conditions where *o*-diphenol oxidases are active during extraction, it also appears to be produced by adding PVX to uninfected but yellowing leaves and re-isolating virus after the leaves have been disrupted in conditions in which the oxidase was inhibited. Modifications other than those that produce PVX-M-like material also occur when PVX is added to leaf extracts containing *o*-diphenol oxidase activity: a form which contains a

https://doi.org/10.23637/ERADOC-1-134

saponifiable and PTBA-reactive fragment, but which readily breaks down to provide subunits has also been observed but not yet satisfactorily characterised.

***PVX structure and reaction* in vitro.** About half of the ϵ-amino groups of the isolated protein of PVX react with pyridoxal 5′-phosphate (PLP) whereas in the intact virus, on average, only one amino group in each protein subunit reacts with PLP. Previous evidence did not make it clear whether this reactive amino group was the same one that reacts with enzymically-generated *o*-quinone, or the ϵ-amino group near the NH_2-terminus of the protein that is readily recognised by proteolytic enzymes such as trypsin. Recent results show that both sites react partially with PLP. PVX-PLP preparations are resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) into two proteins, both of which contain PLP-like fluorescence. One of these proteins is not degraded when PVX-PLP particles are briefly exposed to trypsin, indicating that its trypsin-resistant site is modified by PLP. Estimates of the amount of trypsin-resistant protein in different preparations of PVX-PLP suggest that both sites are modified to approximately the same extent in preparations containing 1 molecule of PLP per subunit, whereas in less pyridoxylated preparations the trypsin-recognition site is more extensively modified than the one that reacts with *o*-quinones.

Five strains of PVX which differ from the one commonly used (X^N) in host range, antigenicity and frequency of occurrence in the field, also react with enzymically generated quinone and PLP without losing much infectivity. Their different behaviour in the field does not therefore depend on different resistance to inactivation by *o*-quinones. Only two strains differ significantly from X^N in the amount of reagent bound. One of these (T^8) binds 1·4 molecules of chlorogenoquinone per subunit at pH 7, and approximately 1 molecule of PLP. About 40% of the subunits in this strain exist in a degraded form, and it is likely that this degradation has exposed another amino group that reacts with quinones but not with PLP. The other strain (X^4) binds 1 molecule of PLP per subunit but only half a molecule of chlorogenoquinone. Its protein is resolved by SDS-PAGE into two components, and only one of these is degraded when virus particles are briefly treated with trypsin. This strain appears to be unique among isolates of PVX in containing two types of protein subunit. Further work is planned to characterise these two proteins and confirm that they are components of the same genome (Ireland, Pierpoint, Strowman, with Carpenter, Plant Pathology Department).

Virus-induced plant proteins associated with resistance. Previous work here (*Rothamsted Report for* 1973, Part 1, 117) and elsewhere has demonstrated that *Nicotiana tabacum* cv. Xanthi-nc can be made resistant to virus infection either by preinoculating with a virus that gives a necrotic or hypersensitive reaction or by injection with polyacrylic acid. This induced resistance is not confined to the preinoculated leaf and is associated with the production of at least four proteins (termed b_1-b_4) which appear as the resistance develops and which decrease in conditions where the resistance disappears. In order to establish whether these proteins have a causal role in inducing resistance or are by-products of the process, we have started to devise methods by which the proteins may be isolated in the native state, characterised and tested for biological activity.

The major b-protein (b_1) has been purified to homogeneity by a procedure involving extraction of the leaf proteins at pH 2·8, gel filtration and chromatography on DEAE-cellulose. Although extraction at pH 2·8 removes a number of contaminating proteins it exposes the b_1 protein to a degree of acidity which could alter its conformation and destroy any biological activity. The procedure was therefore modified to allow extraction of the leaf proteins at pH 8·0 by adding an extra gel filtration as the final step. Yields of

https://doi.org/10.23637/ERADOC-1-134

over 5 mg of purified b_1 protein have been obtained from 200 g of leaf suggesting that it represents about 1% of the soluble leaf protein 1 week after infection with virus.

The u.v. spectrum of purified preparations of b_1 protein were typical of proteins uncontaminated with nucleic acid, E_{280}/E_{260} ratio = 1·84, and the absorbance index ($E^{1\%}_{280\,nm}$) was determined as 18·9. The purified b_1 protein migrates as a single band on SDS-PAGE at pH 7·2 or pH 8·3 in the absence of SDS. The mol. wt. of the b_1 protein by SDS-PAGE was $1{\cdot}5\times10^4$ and by ultracentrifugation was $1{\cdot}4\times10^4$. Its amino acid composition together with its molecular weight suggests that the b_1 protein consists of about 136 residues of which a high proportion are acidic (28%) and aromatic residues (12%) but comparatively few are basic (9%).

The role of the b_1 protein has not yet been defined but its dramatic increase to greater than 1% of the soluble leaf protein suggests its importance in the metabolic state of the plant associated with resistance. These preparations of purified b_1 protein have been tested by other workers for activity against healthy protoplasts, protoplasts infected with TMV and on *in vitro* protein synthesis, but so far no significant effect has been observed. (Antoniw, Norbury and Pierpoint).

Plant-fungal relationships

Fungi of the Gaeumannomyces-Phialophora complex

Isolates of G. graminis. Ten more isolates of var. *graminis* which is not pathogenic to wheat, and three of var. *avenae* (oat take-all) were received from Australia and their behaviour on agar media and in liquid culture has been studied. The *graminis* isolates showed considerable variation in the production of sclerotia and the ability to form perithecia: some formed both, some one or the other and the rest neither. They also differed in their growth in liquid culture, some growing well and others very poorly in the same media. This variety may need to be further sub-divided into strains. Both the *graminis* and *avenae* isolates had little or no polygalacturonase (PGase) activity under any of the conditions tested.

Several methods for the rapid identification of the different varieties of *G. graminis* have been tested. The most useful is to grow the fungi on plain agar incorporating either roots or leaves of oat seedlings. *Tritici* isolates will not grow on either root- or leaf-agar, *avenae* isolates will grow on both media and *graminis* isolates will not grow on the root-agar but grow readily on the leaf-agar.

Cell-wall degrading enzymes. Several isolates of *G. graminis* var. *tritici* (wheat take-all) had widely different carboxymethyl cellulase (CMCase) activity after growth in media with and without carboxymethyl cellulose. The isolate with the highest PGase activity was found to have the lowest CMCase but one isolate with high PGase has high CMCase too. Another isolate has low PGase and CMCase and is the least pathogenic of the isolates in the series tested.

An isolate with high CMCase was tested on other substrates (cellophan, cellulose powder and milled wheat straw) and its cellulase activity was compared with that of representative isolates of the other varieties of *G. graminis* and of *Phialophora radicicola* var. *radicicola* and *P.r.* var. *graminicola* (= *G. cylindrosporus* n.sp.) (Ashby, Holden and Sanders)

Nitrogen metabolism

Intermediary nitrogen metabolism. The Department has a continuing interest in understanding the reactions involved in the formation of organic nitrogen compounds, particularly amino acids and amides, and their transport throughout the plant. The initial

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assimilation of ammonia, whether presented to the plant as such, reduced by the plant from nitrate or fixed in the nodule from dinitrogen gas, takes place via the formation of glutamine and then glutamate and other amino acids. Besides the need for reduced nitrogen, the synthesis of amino acids depends upon the production of carbon skeletons, the formation of which is regulated by feedback mechanisms. Glutamine and asparagine form the major transport compounds in plants and are the chief forms of nitrogen available for developing seeds; consequently, mechanisms for converting the N present in these amides into the full range of amino acids required for protein synthesis must exist in the seeds.

Ammonia assimilation. Last year it was reported that the reaction of nitrite reduction and ammonia assimilation could be measured in intact chloroplasts by monitoring the coupled oxygen evolution; these results have been confirmed by measuring the changes in glutamate and glutamine. Illuminated pea leaf chloroplasts convert nitrite or ammonia to glutamate in the presence of excess 2-oxoglutarate; little glutamine is formed unless 2-oxoglutarate becomes limiting. Ammonia assimilation is blocked by methionine sulphoximine (an inhibitor of glutamine synthetase) and addition of azaserine (an inhibitor of glutamate synthase) causes a build up of glutamine; both of these results are consistent with the operation of the glutamine synthetase/glutamate synthase pathway.

In conjunction with Day and Roughley (Soil Microbiology Department) the rates of dinitrogen fixation and the amounts of the enzymes of ammonia assimilation have been determined in nodules of *Phaseolus vulgaris* during their development. Four varieties have been used in three experiments and the effect of strain of inoculum and the amount of nitrate in the growing medium on the various activities have been investigated. The results show that glutamine synthetase, glutamate synthase and glutamate dehydrogenase are all present in the nodules. The activity of the enzymes correlates with the level of nitrogenase. The effect of nitrate is slightly different in the different varieties but in general it decreases the amount of nodule tissue formed; the rate of N_2 fixation and the activity of the enzymes of assimilation decrease in proportion to the decrease in the amount of nodule tissue.

Enzymes of amino acid metabolism. We have continued to study the biosynthesis of lysine, threonine and methionine. In higher plants these amino acids are derived from aspartate via a branched biosynthetic pathway. The first enzyme in the pathway is aspartate kinase and we have shown, using the isolated enzyme, that it is subject to different control mechanisms in different species. In wheat and maize the enzyme is solely regulated by lysine; in barley lysine is similarly effective, but the inhibition caused by small concentrations of lysine is enhanced by the presence of methionine. Carrot tissue culture contains two isoenzymes, one sensitive to lysine and one to threonine; the relative amounts of the two isoenzymes vary from 1:1 to 10:1 over the period of growth of batch cultures of cells.

The operation of the regulatory mechanisms *in vivo* have been followed by studies on the growth and metabolism of cereal embryos. Growth is synergistically inhibited by lysine plus threonine and this effect is relieved by methionine. Homocysteine and homoserine, biosynthetic precursors of methionine, likewise relieve the inhibition. Methionine biosynthesis *in vivo* was studied by feeding ^{35}S-sulphate to wheat embryos over a period of 8 h. Methionine biosynthesis was inhibited about 50% in the presence of 2 mM-lysine plus 2 mM-threonine at 0·5, 2 and 8 h. This correlates with the 47% growth inhibition caused by these amino acids. Although the results are consistent both with inhibition of aspartate kinase by lysine and of homoserine dehydrogenase by threonine, the observation that methionine synthesis continues at a considerable rate, when there is 95%

https://doi.org/10.23637/ERADOC-1-134

inhibition of threonine synthesis, suggests that there are other regulatory sites governing the biosynthesis of methionine in this tissue.

Subcellular location of aspartate kinase in leaves has been studied and at least half of the enzyme is associated with preparations of pea leaf chloroplasts, separated by differential centrifugation, which were devoid of mitochondrial and microbody marker enzymes. Similar results were obtained with chloroplasts from broad bean leaves. Chloroplasts from pea leaves synthesised ^{35}S-labelled cysteine and glutathione from ^{35}S-sulphate and ^{14}C-labelled aspartate. All of these reactions are greatly stimulated in the light and it seems that the complete aspartate pathway is present in the chloroplasts.

Carrot tissue cultures show a ten-fold increase in the lysine-sensitive isoenzyme of aspartate kinase during part of their growth cycle; however the amounts of lysine, threonine, methionine and isoleucine, both soluble and in proteins, do not alter appreciably during the growth of the cells. The change in isoenzyme levels cannot therefore be correlated with any change in the production of different amounts of the various amino acids of the aspartate family.

A large number of plant pathogenic fungi synthesise lysine via the α-aminoadipic acid pathway. The final intermediate in this pathway is saccharopine and its metabolism by cell free extracts of *Pyricularia oryzae* have been studied. So far we have evidence for three proteins which can catalyse NAD(P)H oxidation in its presence but, it is not certain which one is responsible for its cleavage to form lysine. Unlike saccharopine metabolising enzymes in animal cells, those from *Pyricularia* are not present in the mitochondria.

Amino acid transport and amide metabolism. Analysis of the exudate from the cut stem bases of spring wheat plants grown in the glasshouse showed that the major products of nitrate assimilation from the roots were aspartic acid+asparagine and glutamic acid +glutamine (probably largely in the form of amides). Besides these compounds only alanine and serine contributed significant amounts of N. One notable feature was the absence of cysteine and possibly also of methionine, which implies that the S requirement of the leaves may be met by the transport of sulphate (although no direct determination of this was made). A comparison of the xylem contents of the final internodes subtending the ear with the root exudate suggests that the wheat plant preferentially extracts glutamine and other amino acids, but not asparagine from the xylem stream. A similar relative enrichment with asparagine of the xylem stream as it ascends the plant has been noted in legumes by other workers. The finding that the xylem contents of the final internode are low in proline and glutamic acid+glutamine does not support the hypothesis that the supply of amino acids from the root to the grain, which presumably must occur in the xylem, stimulates the prolamin fraction by providing large amounts of the component amino acids. Comparison of the amino acid composition of the xylem contents of the final internode with that of the harvested grain suggests that the ear itself is capable of synthesising a wide range of amino acids. In addition some of the imbalance may be made up by the export of amino acids from leaves via the phloem. Until suitable techniques for phloem sampling in cereals are available a direct answer to this question is not possible.

Our results for cereals and those of others for legumes suggest that asparagine and, to a lesser extent, glutamine are major sources of nitrogen for developing seeds and that amide-N can be converted in these organs into the range of amino acids required to synthesise the storage proteins. Whilst there is evidence that glutamine may be metabolised to glutamate and thence to other amino acids via the action of glutamate synthase and various transaminases, much less is known of the metabolism of asparagine. In order to study asparagine metabolism during the development of legume cotyledons we have

https://doi.org/10.23637/ERADOC-1-134

utilised the method of Millerd *et al.* (*Australian Journal of Plant Physiology* (1975), **2,** 51-59) to culture these organs. Single cotyledons of developing peas were removed from the pod and cultured in sterile conditions in shaken flasks on a mineral-sucrose medium in the light. Developing cotyledons of *Phaseolus vulgaris* and *Vicia faba* and caryopses of wheat have been cultured under the same conditions. The production of protein by pea cotyledons utilising endogenous amino acids was linear for up to 10 days in culture. The addition of asparagine stimulated protein synthesis up to 3-fold although growth was linear for only 6 days. Maximum rates for protein synthesis obtained with asparagine were 2·4 mg per cotyledon day^{-1}, comparable with the rates achieved in the intact pod. Glutamine supported protein synthesis to a lesser extent, but glutamate was a poor source. The stimulation by asparagine suggests that both nitrogens in the molecule are utilised for synthesis of the other amino acids. Since there appear to be no mechanisms for direct transfer of the amide nitrogen to any keto-acid acceptor it has been suggested that the amide nitrogen of asparagine is liberated as ammonia and then reassimilated via the action of glutamine synthetase and glutamate synthase. This is consistent with the finding that methionine sulphoximine and azaserine both strongly inhibit asparagine-dependent protein synthesis, but the former had no effect on glutamine-dependent protein sythesis and the latter (which inhibited the synthesis of protein from glutamine) had no effect on that from glutamate. The enzyme mechanism by which ammonia is released from asparagine in pea cotyledons is uncertain; asparaginase does not appear to be present and possible alternate pathways are being investigated.

Asparaginase is, however, present in the developing cotyledons of certain species of lupin, and work has continued on the enzyme purified from *Lupinus polyphyllus*. The preparation is apparently homogenous by disc gel electrophoresis, and has a mol. wt. $7{\cdot}2\times10^4$ as determined by sucrose density centrifugation. Analysis on SDS-PAGE suggests that it is a dimer with subunits having a mol. wt. $3{\cdot}8\times10^4$. No significant activation by metal ions could be detected, although it was sensitive to transition elements. Lack of inhibition by mercury compounds suggests that the enzyme does not have a cysteinyl residue near to the active site. An immunological cross-comparison with antisera to plant and bacterial asparaginases and their reactions with plant and bacterial asparaginases (the bacterial antiserum prepared from *Erwinia* was kindly supplied by Dr H. E. Wade, M.R.E. Porton Down) showed that there was no serological cross-reaction between the two enzymes although the *Erwinia* antiserum inhibited the lupin enzyme to a small extent (10%). A survey of the distribution of asparaginase showed that it was present in only five of the 45 species and varieties of *Lupinus* tested; it could not be detected in a wide range of other legumes and cereals. (Awonaike, Bright, Davies, Featherstone, Festenstein, Hill, Hughes, Kirkman, Lea, Leggatt, Miflin, Mills, Norbury, Sodek, Thompson, Wallsgrove and Wood)

Protein synthesis in cereal seeds. We are interested in the way in which a cereal seed lays down its protein store and in determining the factors that affect the quantity and quality of that protein. To do this, we are attempting to (1) identify and characterise the major individual protein components of the seed, (2) locate their sites of synthesis and deposition, (3) identify the messenger RNAs (mRNA) for the major proteins, (4) determine the initiation and duration of the various events of protein synthesis, and (5) understand the factors, both genetic and agronomic, that alter the relative distribution of individual proteins in the grain.

The separation of cereal seed proteins. The protein fractions of cereal seeds were classified by Osborne at the beginning of the century into four groups, depending on their extractibility by different solvents, and were given the general names albumin (water-

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soluble), globulin (salt-soluble), prolamin (alcohol-soluble), and glutelin (dilute acid- or alkali-soluble). The different fractions have specific names in different plants: the prolamin of barley is termed hordein and of wheat, gliadin. This broad classification whilst useful, is not based on a study or definition of the component polypeptide chains of each of the protein fractions. During the past 3 years we have been attempting to define the prolamin and glutelin fractions of barley in terms of their polypeptide composition and to establish techniques which completely extract these component polypeptides with the minimum of cross-contamination. Arising out of an exchange of techniques and joint studies at an EEC workshop in the department at the end of 1976 we have made several improvements in methodology. By our current techniques almost all the hordein and most of the glutelin are extracted with no cross-contamination. The two fractions are characterised by (a) the behaviour of their component proteins in a number of different separation systems: SDS-PAGE at pH 8·9, urea-PAGE at pH 4·6, isoelectric focussing (IEF) in the pH range 5–9 and two dimensional IEF and SDS-PAGE, and (b) their amino acid composition. Hordein polypeptides can be subdivided according to their behaviour on SDS-PAGE and urea-PAGE into three subgroups, A, B and C, of increasing mol. wt. This seems to be justified on genetic grounds since the B and C patterns appear to be closely linked within themselves. It is also possible to define prolamins and glutelins by virtue of their function and location within the seed and work on this is now in progress.

An investigation has started to define the optimum conditions for the extraction of wheat prolamin (gliadin) and glutelin fractions. Different alcholic solvents have been assessed on their ability to extract N from the seed. The results so far show that (a) propan-1-ol is more effective than propan-2-ol or ethanol, (b) more N is extracted in the presence of 1% 2-mercaptoethanol (2-ME), (c) increasing the temperature increases extraction, although the effect differs slightly according to the alcohol used and the presence or absence of 2-ME and (d) addition of 1% acetic acid to propan-1-ol increases the amount of N extracted irrespective of temperature or the presence of 2-ME. Preliminary separation of the fractions on SDS-PAGE suggests that the qualitative nature of the component polypeptides of the extracts varies little between the least effective (70% by vol. ethanol at 4°C) and the most effective (50% by vol. propan-1-ol, 1% acetic acid, 1% 2-ME at 60°C) procedures despite the fact that the former extracts about 15% and the latter 60% of the total seed N.

The identification of cereal varieties by means of their prolamin polypeptide pattern. The polypeptide pattern obtained by separating gliadin on starch gel electrophoresis is widely used in the identification of wheat varieties. This technique is particularly useful because it can be used on single or half seeds. With barley the method is less successful because of the greater difficulty in solubilising and separating hordein. We have now developed techniques for the identification of barley varieties and have evaluated them using flour samples and single seeds. For single seeds hordein is extracted from crushed seeds with 55% (v/v) propan-2-ol+2% 2-ME using ultrasonication at 20°; the extract is then alkylated with vinylpyridine and separated on SDS-PAGE. The patterns obtained are solely dependent on variety and are not affected by environment, nitrogen nutrition, position of the seed in the ear or premature harvesting. A classification of varieties has been constructed based on the 'B' and 'C' hordein polypeptide patterns which appear to be inherited with a degree of independence; there is no apparent variation in the 'A' polypeptide pattern between varieties. Not every variety has a unique pattern but from the 88 varieties studied 29 major groups were distinguished. The largest group could be subdivided into four subgroups using urea-PAGE and no doubt further subdivisions could be obtained by using two-dimensional separation. The method has several ad-

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vantages over existing identification procedures and is of potential importance for the commercial identification of grain samples, as an aid to existing techniques for determining varietal distinctness and in genetic studies. The technique developed for barley revealed minor differences between some wheat varieties which are identical when studied by starch gel electrophoresis.

Biochemistry of developing barley endosperms. A study has been made of changes in the nitrogen fractions of developing seed of the barley cv. Bomi and its high lysine mutant Risø 1508. In Bomi the hordein fraction is synthesised between 2 and 5 weeks after anthesis with only a slight increase between 5 weeks and grain maturity (7 weeks). The hordein fraction represents about half the total endosperm N of the mature grain in Bomi; this is in contrast to Risø 1508 where synthesis is complete at 4 weeks and the final amount of hordein is only 10–15%. The amount of glutelin per endosperm is approximately the same in both varieties and synthesis is essentially complete at 4 weeks. In Bomi the salt-soluble protein fraction accounts for 11–12% of the endosperm N at maturity and synthesis is complete at 3 weeks, whereas in Risø 1508 synthesis continues until 5 weeks and the fraction represents 25% of the final endosperm N. Risø 1508 also has larger amounts of non-protein N from 2 weeks after anthesis to grain maturity and this fraction accounts for 17% of endosperm N compared to 9% in Bomi. It is concluded that the high-lysine character of Risø 1508 does not result from arrested hordein synthesis but from changes in the regulation of protein synthesis which are expressed throughout grain development. Continued studies with *in vitro* protein synthesis systems confirm this and indicate that the regulatory step probably occurs prior to the formation of active polysomes bound to membranes.

Immunology of barley hordeins. As reported last year initial attempts to raise antisera to hordein were unsuccessful. Since part of the problem is due to the formation of precipitation lines in immunodiffusion tests with sera from uninjected rabbits in the presence of SDS, an ^{125}I radioimmunoassay was developed. Further injections of rabbits with hordein samples were made (by Govier, Plant Pathology Department) and two active sera obtained; one from rabbit 74 which had received 5 mg of hordein-2 from Risø 1508 (this fraction consists almost completely of 'B' polypeptides) followed 6 months later by 40 mg of total Risø 1508 hordein and one from rabbit 93 which received 4 mg of 'A' hordein from 1508. Both sera gave positive results in the radioimmunoassay against total Risø 1508 hordein. In the immunodiffusion test the non-specific reaction could be diminished to a weak diffuse line by directly dissolving hordein samples in 0·1% instead of in 1% SDS followed by dialysis against 0·1% SDS even though some hordein was insoluble. Using this modification serum from a rabbit which had received 'A' hordein only gave lines with total and 'A' hordein from Risø 1508 but not with hordein-2 from 1508 ('B' hordein). The other active rabbit serum (74) produced strong lines with total hordein from Risø 1508 and Julia as well as hordein-2 from 1508, down to 1:4 dilution, but only a weak reaction with 'A' hordein. We thus have two antisera for use in subsequent studies, one of which appears to be specific for 'A' hordein and one which, with a small amount of purification, should be specific for 'B' hordein. (Burgess, Byers, Faulks, Festenstein, Hill, Leggatt, Matthews, Miflin, Pratt, Richardson, Sacher, Shewry and Smith)

Staff and visitors

Outside support. The Department is pleased to acknowledge that several organisations have provided funds for the support of personnel and research, particularly the Home

https://doi.org/10.23637/ERADOC-1-134 *pp 9*

Grown Cereals Authority, the Potato Marketing Board, Shell Research Ltd with the Science Research Council, NATO and the EEC. We also have had research contacts with Rank Hovis McDougall and the Burroughs Wellcome Foundation.

Visitors. The Department welcomed a large number of day visitors from many countries and several people spent longer periods. Dr J. W. Anderson and Dr J. Done returned to Australia early in the year and in September Dr L. Sodek on leave from the University of Campinas, Brazil, and Professor J. A. Sacher on leave from California State University, USA arrived to spend a year here. Dr W. R. Mills previously of the University of Miami, Ohio, USA came in September as a NATO postdoctoral fellow. Dr G. E. Edwards from the University of Wisconsin and Professor J. E. Fox, Dr J. Erion and Dr D. Roussell from the Department of Botany, Kansas University, USA spent short periods in the Department exchanging experimental techniques.

Meetings. Members of the department were active in meetings organised by the Society for Experimental Biology, Biochemical Society, the Society for General Microbiology, The Association of Applied Biologists and the British Mycological Society. Papers were given at the International Conference on Nitrogen Metabolism at Long Ashton and at the International Photosynthesis Congress at Reading.

Visits abroad. S. W. J. Bright visited the Danish Atomic Energy Research Station at Risø where he gave a seminar and discussed problems in the use of mutagens and the selection of biochemical mutants. P. J. Lea attended an International Symposium on Nitrogen Metabolism in Plants at the University of California, San Diego, USA, where he presented a paper and he also gave lectures at several other Universities in the USA, and later in the year at the University of Oslo. P. R. Shewry attended the American Society of Plant Physiology Meeting at the University of Wisconsin where he presented two papers and he also spent 2 weeks at the University of Kansas and visited other research laboratories in the USA. W. S. Pierpoint attended the Meeting of the Phytochemical Society of Europe in Ghent, Belgium. B. J. Miflin attended the Kearney Foundation Workshop on Nitrogen in the Environment at the University of California where he presented a paper and he also visited and lectured at various other Universities of the USA. B. J. Miflin and P. R. Shewry both attended the EEC conference at the Justus Liebig University, Giessen, Germany, on Carbohydrate and Nitrogen Metabolism in Plants where they both gave papers.

Students. Three students were awarded their Ph.D. degrees and left for posts elsewhere; M. Wade to the Department of Chemistry, University of Colorado, USA, H. M. Davies to the ERDA/MSU Plant Research Laboratory at Michigan State University, USA, and R. J. Ireland to Queen's University, Kingston, Canada. Sandwich course students working in the Department were P. Richardson, Elizabeth Wood, N. Sanders, Jane Hughes and L. Featherstone. Deborah Thompson continued, and Jayne Matthews started as Ph.D. students. K. O. Awonaike, on leave from the National Cereals Research Institute, Nigeria also joined us as a Ph.D. student working in conjunction with the Soil Microbiology Department. Two CASE studentships, one with the University of Manchester and one with the University of Hull, started during the year.

Staff. Gabriele Loftus resigned as personal secretary and was replaced by Susan Veck. Helen Pratt left for a year's leave of absence to study for a Masters degree and was replaced by Audrey Faulks. Susan Smith was appointed to replace A. Hyde.

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Publications

Book

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Theses

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