ROTHAMSTED RESEARCH

Report for 1976 - Part 1

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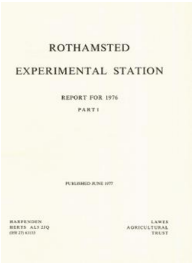

Biochemistry Department

B. J. Miflin

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BIOCHEMISTRY DEPARTMENT

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Introduction

Research in the department can be divided under the main headings: Host/plant pathogen relationships; Nitrogen metabolism of crop plants. A third area of work on tissue cultures, although in some respects separate and reported separately below, is linked to the other topics, particularly nitrogen metabolism.

Host/plant pathogen relationships

Modified forms of potato virus X (PVX)

The aim of this work is to examine the chemical transformations of PVX in leaf tissue, and to see how these affect the infectivity, survival and spread of the virus. The work should also give information on the structure of PVX, and on the chemical modification of plant proteins as they are extracted from tissues rich in phenolic compounds.

Natural modifications. The procedure for isolating PVX from brown senesced leaves has been altered slightly, and it has been confirmed that the PVX in these leaves is modified: it has different u.v. and fluorescence spectra from unmodified PVX, when freeze-dried is grey-brown in colour, but it appears identical to PVX when examined under the electron microscope. On saponification it releases a compound which gives an orange-pink colour (λ max 530 nm and 450 nm) on reaction with sodium periodate-thiobarbituric acid (TBA). Quinic acid, released by saponification of PVX that has combined with chlorogenoquinone *in vitro* (PVX-Q), reacts with periodate-TBA to give a pink colour (λ max 549 nm and 450 nm). Thus, although there are similarities between these two types of modified virus, the TBA-reactive components released by saponification are not identical.

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***Modifications produced by o-quinones* in vitro.** The properties of PVX-Q, made by exposing PVX to chlorogenic acid which is being oxidised enzymically, depend on the pH at which the reaction takes place. Two forms of modified virus, PVX-Q_1 made at pH 7, and PVX-Q_2 made at pH 7·8 have now been examined. The amount of chlorogenoquinone bound to each protein subunit was estimated from the quinic acid released on saponification, and cross linking of the protein subunits examined by gel electrophoresis.

PVX-Q_1, which retains at least $\frac{2}{3}$ of the infectivity of PVX, is grey in colour, and has approximately 1 molecule of chlorogenic acid bound per protein subunit. About $\frac{1}{5}$ of its subunits are cross-linked into dimers and higher polymers. PVX-Q_2 has $\frac{1}{3}$ to $\frac{1}{2}$ the infectivity of PVX, is blue in colour and contains 2 molecules of chlorogenic acid bound per subunit; about $\frac{2}{5}$ of its subunits are cross-linked. Brief exposure to trypsin converts the protein subunits of PVX-Q_1 and PVX-Q_2 to a lower molecular weight form, although the reaction goes less readily and less extensively than it does with unmodified PVX. Prolonged exposure to trypsin releases free quinic acid and, apparently, only one quinic-containing peptide from both forms of PVX-Q.

These results suggest that in PVX-Q_1, predominantly one specific lysine ϵ-amino group has been modified with chlorogenoquinone, and that this is not the lysine residue that is known to be sensitive to trypsin. It is not yet clear if the two chlorogenoquinone molecules attached to each protein subunit of PVX-Q_2 are bound to different lysine residues or linked to each other.

Proteins other than that of PVX have been observed to give blue derivatives on exposure to chlorogenoquinone at pH 7·8.

Modification of different isolates of PVX. A number of different isolates of PVX which affect different varieties of potato, have been examined with respect to their reaction with chlorogenoquinone and pyridoxal-5′-phosphate. So far no striking differences have been observed; thus three isolates bind one molecule of chlorogenic acid per protein subunit at pH 7, become grey-brown in colour and lose only a little infectivity. A fourth isolate reacts similarly but binds about 20% less chlorogenic acid. These differences do not provide an explanation of the host specificities of these isolates or the different frequencies with which they can be isolated from the field. (Giancovich, Ireland and Pierpoint, with in part, Carpenter, Plant Pathology Department)

Plant–fungal relationships

Fungi of the Gaeumannomyces—Phialophora complex

Production of perithecia. Quick and reliable methods for the production of ascospores in culture would be of considerable assistance in the study of fungi of this complex. This has now been achieved with isolates of *Gaeumannomyces graminis* var. *tritici* (wheat take-all) and with var. *graminis* (which causes crown sheath-rot of rice). Isolates of these two varieties which do not form perithecia when grown on standard potato dextrose agar (PDA) medium fruited when PDA was used at quarter strength with the addition of 0·2% yeast extract. Perithecia were also formed on a range of other media, the best being 1% agar containing 0·4% malt extract broth. Of ten *tritici* isolates tested, seven have produced perithecia on two or more agar media and nine have done so by a method recently published by Novotny (*Abstracts of Mycology* (1976), 10, No. 57195). This involves growing the fungus in liquid culture for several days and then transferring the mycelial mat to moist filter paper. On the agar media perithecial initials usually start to appear in about two weeks and ripe ascospores are liberated one to two weeks later. With the Novotny method perithecia were formed in about the same length of time but several isolates failed to produce ripe ascospores. *G. graminis* var. *avenae* (oat take-all) does not seem to form

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perithecia so readily and of six isolates tested only one did so both on agar media and by the Novotny method.

Isolates of *Phialophora radicicola* var. *graminicola* (=*Gaeumannomyces* n.sp.) and var. *radicicola* have been tested on the same range of agar media as used for *G. graminis* and also by the Novotny method but on no occasion have perithecia been observed.

Cell-wall degrading enzymes. The polygalacturonase (PGase) activity of three more isolates of *G. graminis* var. *avenae* has been determined. The results confirm the previous observations with isolates of this variety that the very low activity is not enhanced when pectic substances are present in the medium. Two isolates of var. *graminis* received from Australia differed from the isolate tested earlier which excreted little or no PGase. In one of them a low level of PGase was induced by pectin; the other produced the enzyme in response to the presence of phosphate but not to pectic substances.

Mixed culture experiments in liquid media using pairs of *Gaeumannomyces* and *Phialophora* were continued. Little progress has been made in elucidating the mechanism by which *Phialophora* isolates inhibit the growth of *Gaeumannomyces*. (Ashby and Holden)

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 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. Work has continued on glutamine synthetase and glutamine (amide): 2-oxoglutarate aminotransferase (GOGAT or glutamate synthase), the two enzymes of the primary assimilation pathway for incorporating ammonia into amino acids. Previous work has shown these enzymes to be widely distributed in roots and leaves. Studies in the department and elsewhere have now established that these two enzymes are responsible for the incorporation of ammonia produced by nitrogen fixation in the bacteroids. The bacteroids export ammonia into the plant cytoplasm where glutamine is then formed. Sufficient NADH- and ferredoxin-dependent GOGAT have been detected in the cytoplasm of nodules derived from *Vicia faba* and *Phaseolus vulgaris* to account for the necessary rate of amino acid synthesis within the nodule. Most of the nitrogen leaves the nodule as asparagine, and attempts to measure the formation of asparagine by asparagine synthetase in the nodule are in progress.

Ferredoxin-dependent GOGAT has been further purified from bean leaves using a column of Sepharose to which ferredoxin has been bound. This column specifically absorbs ferredoxin-dependent enzymes which can be eluted with salt and the technique also has potential for the purification of nitrite reductase and ferredoxin antibodies. In the leaves of higher plants both glutamine synthetase and GOGAT as well as other enzymes of nitrogen metabolism are associated with the chloroplast. This has recently been con-

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firmed by following the evolution of oxygen from chloroplasts. Since light-dependent oxygen evolution requires that an associated reduction takes place, it has proved possible, in chloroplasts in which CO_2 fixation has been inhibited by glyceraldehyde, to couple oxygen evolution to both nitrite reduction and GOGAT. Isolated intact chloroplasts have been shown to reduce nitrite and, concomitantly, evolve oxygen at close to the theoretically expected stoichiometry. Illuminated pea chloroplasts also catalyse a glutamine and α-oxoglutarate-dependent oxygen evolution at rates of 8·5 to 13·9 μmol of oxygen mg^{-1} chlorophyll h^{-1}. This is more than sufficient to deal with the rate of nitrogen flux through nitrite reduction. The reaction is specific for glutamine and α-oxoglutarate and is inhibited by azaserine, a specific inhibitor of GOGAT, but not by methionine sulphoximine, a specific inhibitor of glutamine synthetase, nor by aminooxyacetate, a transaminase inhibitor. Chloroplasts, in the presence of inorganic pyrophosphate, ADP and Mg^{2+}, also evolve oxygen on adding of ammonia plus α-oxoglutarate and this is interpreted as being due to the action of glutamine synthetase, forming glutamine which is then reductively transaminated by GOGAT in the oxygen evolution reaction outlined above. The inhibition of oxygen evolution by methionine sulphoximine and azaserine rules out the possibility that photosynthetically coupled glutamate dehydrogenase is involved.

Maize and other plants with the C_4 photosynthetic pathway have two types of chloroplasts, those in the mesophyll and those in the bundle sheath, that have different roles in carbon dioxide fixation. We have studied the distribution of the nitrogen assimilating enzymes in the bundle sheath and mesophyll of maize and found that glutamine synthetase and GOGAT are present in both types of tissue whereas nitrate and nitrite reductases are present only in the mesophyll. The presence of GOGAT in bundle sheath cells is an advantage in conditions in which a large proportion of the nitrogen arrives in the leaves as glutamine. As nitrate and nitrite reductases are not located in the bundle sheath cells, nitrate must be transported to the mesophyll before being further metabolised. This could be related to the large demand for reductant by nitrate and nitrite reductases which is more readily available in the mesophyll cells. Following the transfer of etiolated maize leaves to the light, there is an increase in the isolatable activity of nitrate and nitrite reductases within 15 h. Little alteration in the levels of glutamine synthetase, GOGAT and glutamate dehydrogenase was detected. The induction of nitrate reductase was dependent both upon nitrate and light, but nitrite had no effect on the enzyme level as has been reported previously by other workers. The addition of casein (3% w/v) greatly improves the amount of measurable nitrate reductase extracted from maize tissue, which should be detected in young seedlings even in the absence of added nitrate.

Amide-N metabolism. In an attempt to understand the utilisation of the amide nitrogen of glutamine and asparagine by developing seeds, ^{15}N amide-labelled asparagine and glutamine have been synthesised and fed to the developing ears of rice. The label is incorporated into the storage protein of the grain but the exact location of the label in the individual amino acids is still under analysis.

Work has continued on the highly-purified asparaginase (500-fold) from *Lupinus polyphyllus*. The enzyme is specific for asparagine and a number of closely related analogues but has no action on glutamine or its analogues and is not inhibited by strong inhibitors of glutamine-dependent reactions. It is only present in the maturing seeds of *L. polyphyllus*, no trace could be detected in the roots, stems, leaves or pod. In conjunction with Govier in Plant Pathology, a rabbit was injected with about 1·5 mg of purified asparaginase; serum obtained from this rabbit six weeks later and diluted to $\frac{1}{64}$ produced precipitin lines against the enzyme but no reaction was obtained with control serum even at zero dilution. The asparaginase antibody reaction was also measured using a radioimmunoassay. Using both the assay of enzyme activity in crude extracts and the specific

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antibody techniques, a study was made of the time course of appearance of asparaginase during the development of the cotyledon in *L. angustifolium* var. Unicrop, and the presence of the enzyme in varieties of several *Lupinus* species. The results from both assays were consistent with each other. Asparaginase is most active during the phase of maximum protein synthesis in the cotyledon in *L. angustifolium* but the enzyme is not widely distributed within the range of species tested.

If the amide nitrogen or asparagine is to be utilised, the ammonia liberated during the asparaginase-catalysed reaction must be reassimilated into the α-amino position of amino acids. Both glutamate dehydrogenase and glutamine synthetase are present in the lupin seed at the time of maximum asparaginase activity. Their Michaelis–Menten constants for ammonia were determined as 6·5 and 0·2 mM respectively, suggesting that glutamine synthetase is involved in the assimilation. Both ferredoxin- and NADH-dependent GOGAT were also present allowing for the complete reassimilation of the amide nitrogen of asparagine via ammonia into other amino acids. Low activities of enzymes involved in the transamination of the α-amino group of asparagine to the 2-oxoacids pyruvate, 2-oxoglutarate and oxaloacetate have been isolated from a number of plants. Their precise role in the utilisation of asparagine nitrogen has not yet been elucidated.

Amino acid biosynthesis. Studies have continued on the enzymology of the synthesis of the aspartate family of amino acids, particularly on the first enzyme in the pathway, aspartate kinase. The general properties of the aspartate kinase of wheat seedlings are similar to those reported in *Rothamsted Report for 1975*, Part 1, 22, for the enzyme from barley. Both enzymes demonstrate non-Michaelis–Menten kinetics with respect to aspartate and Mg^{2+}:ATP and are sensitive to inhibition by lysine and its analogue *S*-2-aminoethylcysteine (AEC). The wheat enzyme is more sensitive than the barley enzyme to both inhibitors but, whereas the barley enzyme is also subject to considerable lysine-dependent methionine inhibition, the wheat enzyme is only slightly (5–10%) affected. Neither enzyme is inhibited by methionine on its own nor by threonine, homoserine, isoleucine, leucine, valine nor alanine, either alone or in the presence of lysine. The failure to detect threonine inhibition of the wheat-seedling enzyme, despite many modifications in extraction techniques, is in contrast to the results of other workers on the wheat germ enzyme. It also contrasts with our findings on aspartate kinase from carrot tissue suspension culture. The specific activity of the enzyme preparation from this source is about 10 times greater than that reported from other higher plant sources. Of the aspartate family of amino acids, only lysine and threonine inhibit the enzyme activity and these effects are not enhanced by the presence of methionine. Inhibition by threonine is complete at 1 mM and by lysine at 5 mM. These inhibitions are independent and additive and, from cultures in the middle of their growth phase, about 20% of the total activity is sensitive to threonine, 70% to lysine, and about 10% insensitive to feedback control. However, it has been possible to relate the proportion sensitive to threonine to the period of time since last subculturing the tissue. During the four-week growth cycle, the activity of threonine-sensitive aspartate kinase increases slightly relative to fresh weight over the first seven days and then subsequently slowly declines. In contrast the lysine-sensitive component increases 6-fold in activity during this time and falls back to the starting value after three weeks. These changes are not accompanied by significant alterations in the proportion of aspartate kinase insensitive to feedback control and so represent changes in catalytic activity rather than insensitivity to inhibitors. These results are best explained by the existence of two isoenzymes, one inhibited by threonine and the other by lysine, a situation analogous to the aspartate kinase I and III respectively, of the coliform bacteria. Confirmation of this explanation requires the physical separation of the two activities; affinity chromatography using immobilised lysine and threonine have not been successful, probably because

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of strong non-specific ionic binding superimposed on any specific attachment. Recently, however, a partial separation has been achieved by gel filtration and this is being further developed for improved resolution. The variation in the control of this enzyme between species and within one species at different growth stages is providing valuable information on the fundamental nature of control mechanisms in higher plants, as well as on methods for devising selection strategies for feedback relaxed mutants.

Evidence that the methods of control determined with isolated enzymes actually occur *in vivo* has been obtained by feeding plants with ^{14}C-acetate in the presence of exogenously supplied lysine, threonine and methionine in various combinations. The results have shown that lysine and threonine both regulate their own synthesis and that the combination of the two further decreases threonine synthesis. These results are similar to those previously obtained in carrot tissue cultures and in maize. They confirm the conclusion reached from studies on isolated enzymes that there is no concerted feedback on aspartate kinase.

Higher fungi synthesise lysine via the 2-aminoadipic acid (AAA) pathway. The last enzyme of this pathway is reported to be a fully reversible saccharopine dehydrogenase. This enzyme has been extracted and purified 100-fold from *Pyricularia oryzae* and shown to carry out the reverse, saccharopine-forming reaction from lysine and 2-oxoglutarate. Attempts to demonstrate the forward, lysine-forming reaction have so far proved unsuccessful. Saccharopine dehydrogenase was found to co-purify with 2-aminoadipic-5-semialdehyde-glutamate reductase, the penultimate enzyme of the AAA-pathway. The activities were separated on Bioglass 1500 and Sepharose CL-4B after treating the enzyme extract with 0·1% Triton X-100. Preliminary investigations suggest these enzymes are associated together on a membrane. (Anderson, Bright, Davies, Done, Festenstein, Harel, Hughes, Lea, Miflin, Seneviratne, Shewry, Thomson, Wade and Wallsgrove).

Protein synthesis in cereal seeds. We are interested in determining the way in which a cereal seed lays down its protein store and 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 site 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 and thus change the amino acid composition of the total grain protein, thereby determining its quality.

Separation and characterisation of barley storage proteins. Methods have been developed for the extraction and separation of the prolamin (hordein) and glutelin fractions of barley grain. Grain is milled to pass a 0·5 mm sieve and extracted sequentially with butan-1-ol, petroleum ether (to remove lipids) and 0·5 M–NaCl (to remove salt-soluble nitrogen). Hordein is then extracted by shaking for 3 × 1 h at 60°C in a screw-capped bottle with 55% v/v aqueous propan-2-ol containing 2% v/v 2-mercaptoethanol (10 ml g^{-1} meal). Glutelins are extracted by reduction and alkylation of the seed residue. Extraction of the alkylated glutelin with 70% v/v aqueous ethanol containing 0·7% acetic acid at 60°C gives an 'alcohol-soluble glutelin' fraction which contains a mixture of residual hordein and true glutelin. The 'alcohol-insoluble glutelin', which is soluble in 8 M-urea and 1% sodium dodecyl sulphate at pH 7, contains only traces of residual hordein.

The amount of hordein extracted from normal commercial varieties, such as Julia or Bomi, ranges from 35–50% of the total seed nitrogen. In contrast, the high-lysine mutant Risø 1508 derived from Bomi has only 10–12% of its seed nitrogen in hordein and much higher proportions of non-protein nitrogen, acid alcohol-soluble and insoluble glutelins.

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Reduced and alkylated hordein and glutelin fractions are routinely separated by SDS-polyacrylamide gel electrophoresis at pH 8·9. Improvement in the gel system has greatly increased the resolution of the hordein bands. Comparison of the polypeptide pattern of Bomi and Risø 1508 reveals that the mutant has most of the bands present in Bomi except that one band at mol. wt. $5{\cdot}1 \times 10^4$ represents a much larger proportion of the total.

Attempts have been made to produce antibodies to total purified hordein as well as individual hordein bands. Antisera produced from three rabbits injected in different ways have been tested using double diffusion techniques and, although precipitation bands were produced, they were also present when serum taken from the rabbits before injection, was used. Part of the difficulty may arise because urea and SDS, which are used to solubilise hordein, interfere with antibody–antigen reactions. Alternative techniques for injection of the rabbits and of antibody assay are being tried in the hope of obtaining specific hordein antibodies for various purposes in the study of barley seed protein synthesis.

The biochemistry of developing barley endosperms. Techniques have been developed for the separation of the subcellular components of the developing endosperms of barley and used to compare the variety Bomi with Risø 1508. Both membrane-bound and free polysomes derived from the two lines have been analysed on sucrose density gradients. There is little difference in the profile of the free polysomes, with evidence for the presence of at least six ribosomes per messenger RNA in both varieties. There is, however, some suggestion that the membrane-bound polysomes of Bomi contain more ribosomes than those from 1508. The membrane-bound polysomes support the synthesis of alcohol-soluble, tri-chloroacetic acid-insoluble material that co-electrophoreses with several of the authentic hordein bands on polycrylamide gel electrophoresis. There are differences in the product produced by bound polysomes of Bomi and 1508. Other differences that have been noted between the two varieties are the increased levels of cytochrome c reductase and malate dehydrogenase in 1508, changes in the density and amount of the endoplasmic reticulum and considerable alteration in the appearance, under the electron microscope, of the protein bodies both isolated and *in vivo*.

Protein quantity and quality in cereal seeds. Following last year's confirmation that grain yield and protein composition of wheat is influenced by the relative amounts of N and S supplied externally, wheat was grown at only two levels of applied N representing the extremes of application. The plants were grown, in a growth cabinet under standard conditions, on vermiculite and received all essential nutrients in liquid form; sulphur was withheld from half of each batch of plants after five weeks' growth. The largest yields, as g dry matter of grain per pot were obtained from plants receiving most N, and plants receiving S yielded almost double those grown without. Applying S to the plants receiving little N resulted in a decreased yield of grain, compared with N alone, and smaller grain size.

Analysis of the amino acid composition of the seed protein of wheat growing in the greenhouse under a range of nitrogen and potassium fertiliser has supported previous findings of a decrease in lysine and sulphur amino acids with increasing protein content of the grain. The xylem sap exuding from the cut stem bases of plants in the same experiment was collected at three times during growth and analysed for nitrate and amino acid composition. Although the results have not yet been subject to statistical analysis, most of the nitrogen passing up the plant in the xylem is in the form of amino acids of which glutamine and asparagine are the major components. In general the concentration of lysine is low and decreases with increasing nitrogen fertiliser. The levels of sulphur amino acids in the sap are at the limits of detection by our methods.

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The negative relationship between the percentage of lysine and sulphur amino acids in the protein and the protein content of the seed has also been shown in field-grown barley of the variety Julia. The protein content increased from 8·6% at the lowest nitrogen fertilisation levels (50 kg ha^{-1}) up to 12·6% at 200 kg N ha^{-1}. In contrast, lysine decreased from 4·1 to 3·3 μmol % and cysteine from 2·0 to 1·3%. Hordein was extracted from the samples by the techniques detailed above and found to increase from 36·4 to 49·1% of the total seed nitrogen. The other two fractions, glutelin and salt-soluble nitrogen, showed a corresponding decrease. These changes explain the decrease in percentage lysine composition since the lysine content of the hordein fraction is low (1·0%) and depresses the overall lysine content of the seed. Contrary to the findings of other workers, analysis of the individual polypeptide composition of the hordeins from the different samples did not reveal any differences between the different levels of nitrogen fertilisation. (Burgess, Byers, Festenstein, Fox, Kirkman, Miflin, Pratt, Richardson and Shewry, with Govier, Plant Pathology Department for immunology studies)

Tissue culture studies

The aim of the work is to use techniques of mass culture of plants on controlled growth media to select biochemical variants that have specific desired properties. At present we are seeking plant lines that have lost their internal feedback control mechanisms.

Cereals. Despite the conclusion reached above that lysine and threonine do not act in a concerted manner to inhibit wheat and barley aspartate kinase, they do have a synergistic inhibitory effect on the growth of barley and wheat embryos which is overcome by the addition of methionine. The nature of this inhibition has been investigated by growing barley embryos in varying levels of one of the amino acids whilst holding the other constant. Lysine and threonine behave differently in that the half maximal inhibition value for threonine is relatively unaffected by the amount of lysine present whereas the reverse is not true. The present tentative conclusion is that the inhibition is due to the combined effects of lysine regulation of aspartate kinase and threonine inhibition of homoserine dehydrogenase leading to the plant being starved of methionine. Plants selected for their ability to grow on lysine plus threonine might be expected to have relaxed controls on one of the two enzymes or be unable to take up one or both of the amino acids.

Small plots of seed treated with the mutagen sodium azide were harvested to give 20 kg of the second generation (M_2) seed of Maris Huntsman and Maris Freeman wheat, and Julia and Maris Mink barley for screening with our techniques. The wheat and barley will both be grown on for a further generation (M_3) but the barley is also being screened in the M_2 generation.

Hand-dissected barley embryos from M_2 material, kindly supplied by the Danish Atomic Energy Commission Risø, have been screened for resistance to inhibitory compounds. Two thousand of these embryos have been tested for their ability to grow on 3 mM-lysine + 3 mM-threonine and 7000 for their growth on 0·25 mM-AEC; 22 selections of the first type and 23 of the second have been grown through and produced seed. Embryos of these seeds will now be re-tested for their ability to grow in the presence of the original selection agents.

Carrot tissue cultures. We have moved away from the study of the carrot model system as the work with cereals has progressed. It has been shown that one of the AEC-resistant carrot cell lines retained this resistance after passage through the differentiated plant. Re-investigations of lysine levels in three AEC-resistant cell lines failed to demon-

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strate significant elevation of free-lysine levels. (Bright, Grover, Miflin, Norbury and Wood)

Methodology

Amino acid analysis. Programmes have been developed for the amino acid analyser and related equipment to enable the following analyses to be carried out: (a) routine analyses of seed protein, (b) analyses of alkylated proteins, (c) determination of lysine in fungal spores, (d) separation and quantification of asparagine and glutamine in the free amino acid fraction of plant extracts, (e) in-line counting of radioactivity during separation of amino acids, (f) split stream analyses of amino acids. In the last adaptation one stream is taken for ^{15}N and/or ^{14}C analysis.

^{15}N analysis. Nitrogen gas when suitably excited will emit light in the near u.v. and visible regions of the spectrum. By measuring the intensity of emission spectral lines around 298 nm or 316 nm, it is possible to determine the ^{14}N:^{15}N ratio in the gas. Two spectrometers have been constructed in the last year for these measurements. The nitrogen gas is contained in a sealed discharge tube at about 5 mm Hg total pressure and emission is induced using radiation from a microwave generator. The first spectrometer was made by adapting an Optica CF4DR spectrophotometer to work as an emission spectrometer using the nitrogen-filled discharge tube as the light source. A second totally dedicated spectrometer has since been constructed using a Hilger and Watts D330 double monochrometer. This spectrometer will scan automatically and repetitively the region from 297 to 300 nm and using 4 mm pyrex discharge tubes, can be used routinely to measure the ^{14}N:^{15}N ratio in 1 μg nitrogen gas. By using a helium-xenon carrier, this sensitivity could be increased ten times. (Byers, Hill, Hyde and Leggatt, with, in part, Cornelius and Keys, Botany Department)

Staff

The department has again been pleased to welcome many visitors during the year, several of whom have given seminars: Dr. J. Done, on leave from the University of Sydney, Australia, has worked in the department for a year, and Dr. J. W. Anderson from La Trobe University, Bundoora, Victoria, Australia, for the last six months. Prof. J. E. Fox, Dr. E. Harel and Dr. A. S. Seneviratne returned respectively to America, Israel and Sri Lanka after each spending a fruitful year in the department. In November the department organised an EEC workshop on protein separation techniques, attended by nine people.

Besides attendance by many members of staff at various scientific meetings, Marjorie Byers, M. A. Kirkman and B. J. Miflin gave an invited paper at the 24th Easter School at the University of Nottingham, Sutton Bonington; B. J. Miflin gave an invited paper at the Phytochemical Society Conference in Oxford, and W. S. Pierpoint at an ARC conference on viruses and plant breeding.

Visits abroad have been made by S. W. J. Bright to the International Phytochemical Society meeting on plant cell tissue culture at Munich and to the laboratories of Prof. H. Gaul at Gruenbach, West Germany, and Prof. J. Reinert in West Berlin. B. J. Miflin and P. R. Shewry attended an EEC Plant Protein Programme study group in Risø, Denmark, and P. J. Lea and B. J. Miflin attended the '2nd International Symposium on Nitrogen Fixation' at Salamanca, Spain, where they presented a paper.

During the year J. Antoniw joined the staff from the University of Dundee; Monica Austin resigned to accompany her husband to East Malling and was replaced by Gabriele Loftus; and A. Hyde also resigned.

Sandwich course students working in the department were: R. Grover, S. Kalaher, Jane Hughes, Elizabeth Wood, P. Richardson.

Deborah Thomson joined the department as Ph.D. student.

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Publications

General Papers

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