ROTHAMSTED RESEARCH

Report for 1975 - Part 1

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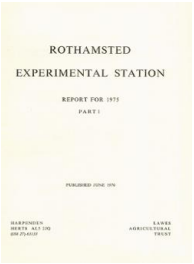

Biochemistry Department

B. J. Miflin

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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. The rationale for the individual topics is given as a short introduction under each heading.

Host/plant pathogen relationships

Any particular combination of a host and its pathogen will interact both on a physical and chemical level. A knowledge of the chemical interaction should provide answers to the questions—how does the pathogen degrade the cell wall to obtain entry? What are the vital components obtained by an obligate parasite from its host? What is the effect of various 'defence' reactions of a host on the pathogen?—and will increase our knowledge of the disease, and may suggest control mechanisms.

Modified forms of potato virus X (PVX)

Natural modifications. Based on the properties of PVX modified *in vitro* by reaction with chlorogenoquinone (PVX-Q), a method has been devised for extracting modified virus from infected senescent tobacco leaves. The leaves are extracted in buffered diethyl dithiocarbamate solution and the extracts clarified with chloroform, concentrated by pressure dialysis and centrifuged in a density gradient of buffered sucrose. The method has, however, proved only partly successful. When unmodified virus was added to healthy green leaves, or to fully shrivelled leaves, it could be re-isolated unchanged, but PVX re-isolated from partly senescent leaves was modified even though no polyphenoloxidase could be demonstrated in the extracts. The procedure therefore needs alteration to prevent changes occurring during extraction from partly senesced leaves. Nevertheless, it has given preliminary indications that quinone-modified virus occurs in, and can be extracted from, fully senesced infected leaves.

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***Modification produced by o-quinones* in vitro.** PVX is modified during the enzymic oxidation of caffeic acid. The reaction appears to go more extensively than with chlorogenic acid and gives a more highly modified product which is uninfective. This material, unlike PVX-Q, is absorbed on to porous glass, and cannot be easily separated into protein and nucleic acid. Although preparations can be made which are modified but still infective, it is not yet clear if their infectivity is due to residual unmodified PVX.

Pyridoxal phosphate derivatives. Previously summarised evidence suggested that pyridoxal phosphate (PLP) reacted with one of the lysine ϵ-amino groups in each protein subunit of PVX, and that this amino group may be one of those which reacts with *o*-quinones. Because the evidence relied heavily on spectrophotometric estimations, more direct chemical substantiation was sought.

The initial complex formed between PVX and PLP is reversible and it absorbs light at 420 nm. When it is reduced with tritiated $NaBH_4$, the viral protein becomes radioactive and fluoresces at 325 nm. There is no evidence for any reaction with the viral RNA. Hydrolysis of the isolated protein in 6N-HCl produces a compound which migrates at the same rate as synthetic pyridoxal ϵ-lysine in three chromatographic solvents and two electrophoretic ones. Its absorption and fluorescence spectra are the same as the synthetic material. Although this confirms that the virus-bound PLP is attached to lysine ϵ-amino groups, there is an indication that it is not attached to the same amino group in each subunit of the virus. Electrophoretic separation of the peptides produced by digesting pyridoxylated protein with trypsin reveals the presence of at least two fluorescent peptides.

PLP reacts more extensively with protein isolated from the virus than it does with the protein of the intact virus. Trypsin digestion of the modified protein produces two fluorescent peptides not produced from the protein of modified virus. Chemical estimates suggest that five or six of the amino groups are modified, and these estimates are consistent with UV absorption and fluorescence measurements. Thus three to four amino groups which are capable of reacting with PLP are unavailable or hidden when the protein subunits are aggregated into the virus helix. (Ireland and Pierpoint)

The trapping of o-quinonoids with benzene sulphinic acid. Benzene sulphinic acid is often used to trap transient quinonoid compounds. Anomalies in the reported properties of the resulting sulphones, suggested that the reaction does not always give the same products and prompted us to re-examine the sulphones formed from the quinones of chlorogenic and caffeic acids in different conditions.

Sulphones produced during the enzymic oxidation of these acids by tobacco leaf polyphenoloxidase have, according to their NMR spectra, sulphur substituted into the 6′ position of the aromatic nucleus. By contrast, sulphones produced in tetrahydrofuran solution from chemically synthesised caffeoquinone and its methyl ester, are substituted at the 5′ position. However, when synthetic caffeoquinone reacts with the sulphinic acid in aqueous buffer, the 6′ sulphone, identical with that produced from enzymically oxidised caffeic acid, is formed. Thus the solvent 'directs' the course of the reaction, possibly by determining whether quinone or semiquinone is the predominant reactive species. (Pierpoint, with Dr. R. Davies of the Food Research Institute, Norwich)

Plant-fungal relationships. A study of cell-wall degrading enzymes of fungi of the *Gaeumannomyces-Phialophora* complex has continued. Of 17 isolates tested only those of wheat take-all, *G. graminis* var. *tritici*, produced large amounts of polygalacturonase (PGase) in liquid media in response to the presence of pectic substances. Three isolates of oat take-all, *G. graminis* var. *avenae*, one of *G. graminis* var. *graminis*, which is pathogenic to rice, and four of the non-pathogenic fungus *P. radicicola* var. *graminicola*,

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excreted little or no PGase with or without pectin present in the medium. *P. radicicola* var. *radicicola*, which is likewise non-pathogenic, produced small amounts of PGase when both pectin and phosphate buffer were present in the medium, but this was less than 10% of the PGase activity of *G. g. tritici* isolates grown under the same conditions. Thus the results obtained show only a partial correlation between pathogenicity and the production of PGase.

The presence of *P. r. graminicola* is known to restrict the growth of *G. g. tritici* and *G. g. avenae* on roots. Recently, Wong (*Soil Biology and Biochemistry* (1975), **7**, 189–194) found that *G. g. graminis* has a similar effect in diminishing infection by both these varieties. The mechanism for this is not understood so the effects of growing *G. g. tritici* in mixed cultures with other isolates are being investigated. *P. r. graminicola* is slow-growing and when the medium is inoculated with this at the same time as the *G. g. tritici* the latter grows fairly well and produces PGase. But when *G. g. tritici* is added to a medium in which *P. r. graminicola* has started to grow, the growth and PGase production of *G. g. tritici* are diminished, the extent depending on the time the *P. r. graminicola* has been growing. *G. g. tritici* does not grow at all when *P. r. graminicola* is given a start of five days. When the mycelium from a *P. r. graminicola* culture is filtered off and the filtrate sterilised either by autoclaving or by passing it through a sterilising membrane, the *G. g. tritici* grows well in it and PGase production is normal. If the medium is not sterilised the *P. r. graminicola* re-grows from many small centres and growth of *G. g. tritici* is inhibited. Preliminary experiments with *G. g. graminis* and *P. r. radicicola*, which grow at similar rates to *G. g. tritici* suggest that they have less effect than *P. r. graminicola* on the growth of *G. g. tritici* in liquid media. (Holden)

Cell walls of coleoptiles and roots of young wheat seedlings have been isolated by pectin standard procedures. Reliable determination of the uronic acid content of root pectin was difficult, but preliminary results show that whereas the pectin content of coleoptiles is 1–2% of the dry weight of the wall, that of the roots is less than 0·4%. (Festenstein)

Nitrogen metabolism

Intermediary nitrogen metabolism. The work is directed towards an understanding of the transport and transformation of intermediary nitrogen compounds within crop plants. We are particularly concerned with the reactions involved in the initial assimilation of inorganic nitrogen into the organic form. This takes place chiefly through the formation of glutamine. Glutamine and its related amide, asparagine, form the major transport compounds of nitrogen in crop plants, and are the chief forms of nitrogen available to the developing seed. We are interested in the way in which the nitrogen in these amides is utilised to form other amino acids for protein synthesis. At present we are also interested in the nature and regulation of the synthesis of the two nutritionally-limiting amino acids, lysine and methionine.

Nitrogen assimilation. Evidence has continued to accumulate that the primary route of ammonia assimilation (whether the ammonia arises from N_2 fixation, NO_3^- reduction or direct uptake) is via the combined action of the enzymes glutamine synthetase and glutamine(amide):2-oxoglutarate aminotransferase (GOGAT). It now seems unlikely that glutamate dehydrogenase plays a major role in the process.

GOGAT has been purified 100-fold from field bean (*Vicia faba*) leaves; it has a molecular weight of 145 000 and a high affinity for its substrates α-oxoglutarate and glutamine. The enzyme has been found in every tissue so far examined. In leaves it is most active in pea chloroplasts (4·6 μmol mg^{-1} Chl, h^{-1}) followed by those of bean, maize and barley. The leaf enzyme is ferredoxin-dependent, and where tested (pea and bean) it does not

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function with reduced pyridine nucleotides. Asparagine cannot substitute for glutamine as an amide-amino donor and no other keto acid can replace α-oxoglutarate as an acceptor.

GOGAT has also been found in the roots of pea, rice, barley, maize and field bean, and the enzyme from pea has been studied in most detail. In contrast to the leaf enzyme, that from roots can accept electrons from NAD(P)H as well as from ferredoxin. Dougall (*Biochemical and Biophysical Research Communications* (1974), **58**, 639) and Fowler *et al.* (*FEBS Letters* (1974), **46**, 340) have claimed that GOGAT from non-green tissues can accept asparagine as a nitrogen donor, and this has been investigated. Asparagine, like glutamine, was found to stimulate the oxidation of NADH in the presence of α-oxoglutarate, but this reaction differed from the glutamine reaction (Miflin & Lea, *Biochemical Journal* (1975), **149**, 403–409). Eventually the cause of NADH oxidation was traced to the presence of aspartate contaminating commercial asparagine preparations. Aspartate in the presence of α-oxoglutarate and aspartate transaminase is converted to oxaloacetate, which in the presence of malate dehydrogenase oxidises NADH. Purified asparagine does not stimulate NAD(P)H oxidation and is not a substrate for pea root GOGAT. Further studies established that the NAD(P)H oxidation observed was a result of a true GOGAT reaction.

A ferredoxin-dependent GOGAT has been found in cotyledons of developing lupin seeds, and a NADH- and ferredoxin-dependent one in developing barley endosperm. The enzyme is important during seed development in the utilisation of glutamine in the transport stream and, together with glutamine synthetase, in the reassimilation of any ammonia liberated from asparagine (see below).

The distribution of some enzymes of nitrogen metabolism in the mesophyll and bundle sheath cells of maize leaves has been studied. Nitrite reductase is restricted to the mesophyll cells, while GOGAT and glutamine synthetase are found in both tissues. GOGAT activity on a chlorophyll basis is higher in the bundle sheath than the mesophyll cells. The two tissues differ in their ratio of transferase to synthetase activity of glutamine synthetase.

Amide-N metabolism. Half the nitrogen in glutamine and asparagine is in the amide position and must be transferred to the α-amino position to form other amino acids. GOGAT, which has been found in developing cereal and legume seeds, can catalyse the formation of amino acids from the amide nitrogen of glutamine. No comparable enzyme has been found that catalyses the transfer of the asparagine amide group, although this compound may form up to 70% of the total nitrogen transported in legumes. Other possible enzymes that can use asparagine are asparaginase and asparagine transaminase. Asparaginase has been purified 200-fold from the developing cotyledons of *Lupinus polyphllus*. This enzyme hydrolyses asparagine to aspartate and ammonia; the liberated ammonia must be reassimilated with the expenditure of ATP. Enough asparaginase is present to explain the breakdown of asparagine in this species of *Lupinus*, but we have so far been unable to find the enzyme in other legumes and doubt whether it is a generally applicable pathway.

Amino acid biosynthesis. Studies on the biosynthesis of members of the aspartate family of amino acids, particularly lysine and methionine, have continued. Aspartate kinase has been extracted and partially purified from barley seedlings. Enzyme activity is dependent on ATP, aspartate and Mg^{++} or Mn^{++}. There is a complex relationship between activity and aspartate concentration, which is difficult to interpret in terms of Michaelis-Menten kinetics. However, by varying the aspartate concentration between 0 and 2 mM an apparent K_m of $\simeq$ 3mM was obtained. The enzyme was subject to allo-

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steric feedback control by lysine and methionine. Lysine alone caused about 35% inhibition at 0·5 mM, and almost complete inhibition at 5 mM. *S*-2-aminoethyl-L-cysteine (AEC), an analogue of lysine, is also an inhibitor but a high concentration is required and the inhibition is incomplete (35 and 74% at 5 mM and 10 mM respectively). Inhibition by lysine and AEC are both competitive with respect to aspartate. Methionine causes co-operative feedback inhibition in the presence of lysine, but is inactive on its own; the addition of 0·5 mM methionine to the same concentration of lysine results in an increase from 35 to 70% inhibition. Methionine also increases inhibition caused by AEC. Other amino acids (including threonine, leucine, isoleucine, homoserine and valine) have no effect either singly or in the presence of lysine.

Aspartate kinase has also been partially purified from carrot tissue cultures. This enzyme has many similar properties to the barley enzyme, although it appears to differ in its allosteric feedback characteristics. Preliminary results indicate that the enzyme is inhibited by relatively high concentrations of lysine, and to a lesser extent by threonine, but no evidence of cooperative effects between lysine and threonine, or lysine and methionine, have so far been found.

Parallel studies on amino acid synthesis *in vivo* have been carried out with carrot tissue cultures, and maize and field bean leaves, by studying the incorporation of radioactive precursors into both soluble and protein pools of amino acids. In tissue cultures threonine and lysine, supplied separately, selectively inhibit incorporation of ^{14}C-acetate into protein threonine or lysine by 60% and 70% respectively. No further increase in the inhibition of ^{14}C incorporation into lysine was observed when lysine and threonine were added together, but an increased inhibition of threonine synthesis was noted. AEC selectively decreased total incorporation into lysine by 20%, although ^{14}C in the soluble pool was increased to 150%. This result is probably due to inhibition of lysine incorporation into proteins by AEC. The flow of ^{14}C into lysine and threonine was also inhibited by 1,5-diaminopentane and 1,6-diaminohexane. While the results are complicated by some inhibition of total protein synthesis, they are compatible with *in vivo* feedback inhibition of aspartate kinase by lysine and of homoserine dehydrogenase by threonine. The results obtained with maize are consistent with the previously published data on maize aspartate kinase; notably lysine synthesis is only inhibited by lysine and is not inhibited by threonine, either on its own or in the presence of lysine. In fact threonine increases ^{14}C-acetate incorporation into the total lysine pool, probably due to the inhibition of homoserine dehydrogenase.

The fate of ^{35}S-cysteine fed to detached leaves of field bean (*Vicia faba*) is being studied. Cysteine was rapidly taken up from 10^{-2}M-cysteine solution, and radioactivity was detected in the lamina within 2 min of the start of feeding. Feeding was continued for 1 and 2 h and the leaves extracted with 80% ethanol and the extract chromatographed and electrophoresed. The major radioactive components of the extract were cysteic acid and an acidic compound that was ninhydrin negative. Between 10 and 20% of the ^{35}S was recovered in cysteine and less in methionine; only very small amounts were recovered in a compound that had the characteristics of cystathionine. When homoserine or *o*-phosphohomoserine, synthesised by homoserine kinase extracted from yeast, were fed together with cysteine, much smaller amounts of cysteic acid and the unidentified acidic component were found and larger amounts of radioactivity were coincidental with cysteine and methionine.

The final enzyme in the synthesis of lysine in plants (diaminopimelate decarboxylase DAPDC) and in higher fungi (saccharopine dehydrogenase) have both been studied. DAPDC has been demonstrated in several plants and partially purified from potato tubers and bean leaves, and some of its properties determined. In attempts to determine the K_m it was found impossible to saturate the enzyme with substrate (similar to the

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observations on aspartate kinase) suggesting that the enzyme probably also exhibits negative cooperativity with increasing substrate concentration. The enzyme is slightly stimulated by pyridoxal phosphate and is inhibited by aminooxyacetate. The results are consistent with *in vivo* formation of lysine by this enzyme in higher plants. In contrast most fungi form lysine by a different pathway, and we have shown that *Pyricularia oryzae* is incapable of synthesising lysine from ^{14}C-labelled diaminopimelate, but can form it from ^{14}C-α-aminoadipate. Although *P. oryzae* converts exogenously supplied saccharopine to lysine *in vivo*, we have not observed the enzymic conversion *in vitro*. Saccharopine dehydrogenase is normally assayed by measuring the formation of saccharopine from lysine. We have partially purified an enzyme that carries out this reaction from *P. oryzae* and determined some of its properties. When saccharopine is supplied to this enzyme in the presence of NADH, the latter is oxidised; however the major product of the reaction is not lysine but α-aminoadipic semialdehyde. There is no evidence that the enzyme we have isolated can synthesise lysine. Further studies are being made to isolate the enzyme responsible for lysine synthesis in this fungus.

Several of the enzymes of nitrogen metabolism are localised in the plastids. Most of the leaf DAPDC and homoserine dehydrogenase is present in intact chloroplasts. The regulatory properties of the latter enzyme have been studied and the bean chloroplast enzyme shown to be particularly sensitive to feedback control by threonine.

Non-protein amino acids. Aqueous ethanolic extracts of seeds of over 40 species of tropical legumes collected during the Zaire River Expedition have been screened for new non-protein amino acids. Several compounds have been selected for further investigation. Two diastereoisomers of 4,5-dihydroxypipecolic acid were isolated and identified by co-chromatography and NMR spectroscopy against authentic standards. They are 2(S)-carboxy-4(R),5(R)-dihydroxypiperidine from *Julbernardia paniculata* and 2(S)-carboxy-4(S),5(S)-dihydroxypiperidine from *Isoberlinia tomentosa*. Although the latter compound has previously been isolated from *Derris*, it is believed that the former compound has not previously been found in plants.

Higher plants frequently contain 5-oxoproline (pyrrolidone-5-carboxylic acid), and although it can be formed as an artifact of extraction it is also a product of various enzyme activities. Catabolism of ^{14}C-5-oxoproline in a number of plants has been studied and its conversion to glutamate and glutamine observed. Using methionine sulphoximine as an inhibitor of glutamine synthetase, the results suggest that the initial product of 5-oxoproline metabolism is glutamate. Whether this conversion is related to the operation of the γ-glutamyl transport system of amino acids, as postulated in animal livers, is not yet known.

In collaboration with Dr. C. Potter of Burroughs Wellcome, Berkhamsted, a number of analogues of glutamic acid have been tested for their action on insect neuromuscular junctions; γ-methyleneglutamic acid, which was isolated from peanuts (*Arachis hypogaea*), has been shown to be a very potent transmitter. (Bryan, Davies, Fowden, Harel, Hill, Lea, Mazelis, Miflin, Pratt, Shewry, 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.

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Separation and characterisation of barley storage proteins. Techniques for the isolation and separation of the individual polypeptides of the storage proteins of barley have been developed. Barley seeds were extracted following a modified Osborne procedure, and the various fractions further sub-divided by gel electrophoresis.

The chief aim has been the characterisation of hordeins. Much of the hordein can be extracted by 55% propan-2-ol but a further fraction is released when mercaptoethanol is added. During sodium dodecyl sulphate (SDS) electrophoresis at pH 8·5, hordein is separated into four distinguishable bands; the hordein released by mercaptoethanol is particularly rich in one of these bands. Hordein can also be fractionated by gel electrophoresis in urea-containing buffer at pH 4·6. The two procedures have been combined to give a two-dimensional gel electrophoretogram showing that some of the bands in SDS consist of more than one protein. Further studies on the resolution of the individual bands are being made to obtain pure samples for amino acid analysis and other characterisation. The composition of the glutelin fraction of barley also has been studied, particularly by making cyanoethyl and pyridylethyl derivatives of the protein, thus preventing the reaggregation of the peptide chains via sulphydryl bonds after extraction. Although extraction of this protein fraction without partial hydrolysis is extremely difficult and the ideal technique has not yet been achieved, preliminary results show that it contains some of the bands that are seen in the SDS electrophoresis of hordein. The composition of the storage protein of the high lysine mutant Risø 1508 has been compared with its parent variety Bomi. The hordein content of the mutant is very low, and it is enriched in one of the polypeptides that is released by mercaptoethanol.

The components of the protein synthesising system. Work has begun on the characterisation of the biochemical machinery of storage protein deposition in developing barley seeds. A comparison between the high lysine mutant 1508 and its normal parent was also made. Polyribosomes have been isolated from developing seeds and shown to incorporate ^{14}C-amino acids into trichloroacetic acid-precipitable material (protein). When these products are separated by SDS electrophoresis there are streaks of activity coelectrophoresing with authentic hordein. Current work is aimed at isolating the hordein message and translating it completely *in vitro*.

Separation of the subcellular organelles of normal and high lysine barley has been achieved by differential and sucrose density gradient centrifugation. Obvious differences exist between the two, chiefly a much altered behaviour of the endoplasmic reticulum and an absence of protein bodies in 1508. The ability of the polysomes, derived from the endoplasmic reticulum, to incorporate amino acids into protein also differs.

Factors affecting protein synthesis. Analyses of wheat seeds derived from plants fed a range of amino acids in the xylem stream (*Rothamsted Report for 1974*, Part 1, 22) has not provided any evidence for the suggestion that altering the composition of the amino acids in the xylem sap affects the amino acid composition of the storage proteins. This may be due to several factors, including the ability of the developing ear to metabolise the amino acids to other compounds and the inability to feed sufficient amino acids to alter by more than 30–40% the amount of any particular amino acid in the endosperm. Support for both these possibilities has been obtained. We have shown that ^{14}C-lysine fed to individual florets or to whole ears of barley is degraded to a number of compounds including α-aminoadipic acid, pipecolic acid, glutamate and proline. Other workers have shown that for legumes most of the nitrogen flow to the seed occurs in the phloem. At present we have no techniques for sampling the phloem.

Despite these negative results, we have confirmed that agronomic factors can influence considerably the amino-acid composition of cereal grains. Our continued study of the

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amino-acid composition of sulphur-limited wheat plants produced by Bolton in the Chemistry Department (see Chemistry Department, p. 96) confirms that the level of cyst(e)ine and methionine in the grain is dependent on an adequate sulphur supply, particularly when large amounts of nitrogen are applied. In these experiments the sulphur depletion has usually been sufficiently severe to affect the yields of grain. However, when field-grown material from Broadbalk and Woburn in 1972 was analysed, it was also found that the level of cyst(e)ine in the grain protein decreases as the amount of nitrogen applied increases. The percentage of protein in the grain and the yield of grain, except at the highest nitrogen fertiliser levels, are increased under these conditions. Analysis of the lysine content of the protein of the same samples shows that it also is negatively correlated with the percentage of protein in the grain. The absolute values obtained for lysine and cyst(e)ine in these analyses appear to be affected by the previous cropping history of the plot from which they were derived.

Analyses of the protein from wheat derived from pot experiments carried out by Talibudeen in Chemistry in which the timing and amount of both nitrogen and potassium fertilisers has been altered, confirms the basic finding that lysine content is negatively correlated with protein content of the grain. The effects of potassium are a little more difficult to explain without further analysis, although the generally negative relationship between cyst(e)ine content and increasing protein still holds.

Developmental work. Much time has been spent on setting up and standardising a new Technicon amino acid analytical system. We now hope to have a much more sophisticated and flexible means of analysing amino acids from different materials. A 2-h programme for the two column analysis of protein hydrolysates, with particular reference to lysine, cyst(e)ine and methionine, has been devised and is now in routine use. Current work is directed towards a better separation of the basic amino acids. Using a one column system with automatic temperature programming and a lithium buffer gradient, asparagine and glutamine have been separated and quantitatively determined, making it possible to determine simultaneously the amide and amino acid content of non-protein fractions.

In collaboration with Keys and Cornelius of the Botany Department, work has commenced on the development of an emission spectrophotometer for ^{15}N analysis. An Optica CF4DR spectrophotometer has been adapted to measure the emission spectrum of ^{15}N in an RF field, and a purpose built emission spectrophotometer is being constructed using a Hilger and Watts D330 monochromator.

Commercially available ^{15}N amide-labelled glutamine and asparagine are prohibitively expensive. We have, therefore, developed techniques for the synthesis of both of these compounds, and have so far succeeded in synthesising ^{15}N amide-labelled asparagine. (Byers, Charlton, Fox, Hill, Kirkman, Lea, Miflin, Pratt, Seneviratne and Shewry)

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 regulation of the synthesis of lysine and methionine, and are thus over-producing these amino acids. However, once established, the techniques can be applied to other ends. The chief requirements are: that a suitable selection technique can be devised; that a culture method is available that allows us to grow the large number of individual genomes for screening; and that we can derive differentiated plants from any selected individuals.

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Selection studies. We have continued to analyse carrot cell lines, selected last year, for levels of lysine. Two lines resistant to AEC and one resistant to DL-α-aminocaprylic acid (ACA) have modest two- to three-fold increases in the levels of free lysine. Two of these have been shown to be much less sensitive to growth inhibition by lysine plus threonine than the normal carrot cells. Plants have been regenerated from the three lines above to see if there is a genetic basis for the increased lysine levels. A number of carrot cell lines resistant to selenomethionine (a methionine analogue) have been isolated and will be screened for excretion of methionine.

Culture of plant tissues. Barley tissue cultures from apical meristems maintain their ability to differentiate plants for some months in callus cultures but will not form liquid suspension cultures. Alternative systems have been tested for selecting for biochemical mutants of barley. Anther culture provides a potential source of large numbers of haploid genotypes, but available techniques allowed only a tiny fraction of these to be stimulated to divide in culture (at best an average of 8–10% of anthers plated produced one or a few calluses). Most of the plants regenerated from anther derived calluses were albino.

Large numbers of embryos have been isolated from dry seed of wheat and, less successfully, barley. These embryos when plated under sterile conditions on a medium containing 0·2 mM AEC or a combination of 1 mM each of lysine and threonine are prevented from growing. The inhibition can be relieved by the addition of lysine and methionine respectively. This system will be used on a larger scale to screen embryos for over-production of lysine and methionine. Embryos will be prepared from the progeny of plants growing at Rothamsted from seeds treated with the mutagen sodium azide.

Epicotyl segments from field bean seedlings have been induced to form roots but not shoots. The callus which is formed from epicotyl or leaves is hard and will not form liquid suspensions. Liquid suspension cultures have been initiated from root segments. Single cell cultures have also been prepared by isolating leaf mesophyll protoplasts. These can be kept alive for two weeks and will synthesise a new cell wall. No continuing cell division has yet been observed. (Bright, Grover, Miflin and Restall)

Staff

During the year the department has had many visitors, particularly from overseas. Professor J. E. Fox from the University of Kansas, USA, Dr. E. Harel from the Hebrew University of Jerusalem, Israel, and Dr. A. S. Seneviratne from the University of Sri Lanka, are working in the department for one year. Dr. J. K. Bryan from Syracuse University, USA, spent three weeks here under the auspices of the Underwood Fund, giving seminars and working in the laboratory. Numerous other people visited for the day and gave seminars. Professor M. Mazelis returned to the University of California, Davis, USA, after a characteristically colourful year in the department. The effect of these comings and goings has been to give the department a valuable source of stimulation and challenge.

Members of the department have attended various scientific meetings, organised by the Biochemical Society, Phytochemical Society and the Society for Experimental Biology. S. W. J. Bright spent four weeks at the John Innes Institute, Norwich, working on anther cultures of barley with R. Chaleff. P. J. Lea visited the USA for three weeks and attended and gave two papers at the American Institute of Biological Sciences meeting at Corvallis, Oregon. B. J. Miflin gave an invited paper at the International Potash Institute Colloquium on Fertiliser Use and Protein Production in Denmark, and was invited to participate in an International Conference on Crop Productivity in Michigan, USA.

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P. J. Lea, originally a Royal Society Research Fellow in the department, has now joined the permanent staff. Monica Newman has changed her name upon marriage to D. J. Austin (CLU). C. Restall, M. Charlton, R. Grover, S. Kalaher, and S. Smith, have worked in the department as sandwich course or vacation students.

Publications

General Papers

1 Fowden, L. (1974) Non-protein amino acids from plants: Distribution, biosynthesis and analogue functions. *Recent Advances in Phytochemistry*, vol. 8, Metabolism and regulation of secondary plant products. Ed. V. C. Runeckles and E. E. Conn, New York: Academic Press, pp. 95–122.

2 Holden, M. (1975) (Editor) Guide to the literature for the identification of British fungi (3rd Edition). *Bulletin of the British Mycological Society* **9,** 67–106.

3 Holden, M. (1976) Chlorophylls. In: *Chemistry and Biochemistry of Plant Pigments* (2nd Edition) Ed. T. W. Goodwin, New York: Academic Press, Vol. 2, pp. 1–37.

4 Lea, P. J. & Fowden, L. (1975) Asparagine metabolism in higher plants. *Biochemie und Physiologie der Pflanzen* **168,** 3–14.

5 Lea, P. J. & (Norris, R. D.) (1976) Amino acid analogues in biological studies. *Science Progress, Oxford* **63,** 65–85.

6 Miflin, B. J. (1976) Potential for improvement of quantity and quality of plant proteins through scientific research. *Proceedings of the 11th IPI Colloquium: Fertiliser use and protein production*, pp. 53–74.

Research Papers

7 Bright, S. W. J. & (Northcote, D. H.) (1975) A deficiency of hypoxanthine phosphoribosyltransferase in a sycamore callus resistant to azaguanine. *Planta* **123,** 79–89.

8 Byers, M. (1976) Relationship between total N, total S and the S-containing amino acids in extracted leaf protein. *Journal of the Science and Food and Agriculture* **27,** 135–139.

9 Byers, M. (1976) Determination of S in extracted leaf protein. *Journal of the Science and Food and Agriculture* **27,** 131–134.

10 (Cheshire, R. M.) & Miflin, B. J. (1975) The control of lysine biosynthesis in maize. *Phytochemistry* **14,** 695–698.

11 Lea, P. J. & Fowden, L. (1975) Glutamine dependent asparagine synthetase from *Lupinus albus*. *Proceedings of the Royal Society B* **192,** 13–26.

12 Lea, P. J. & Miflin, B. J. (1975) The metabolism of asparagine in the roots and leaves of *Pisum sativum*. *Plant Physiology* **56,** Supplement, p. 66.

13 Lea, P. J. & Miflin, B. J. (1975) The occurrence of glutamate synthase in algae. *Biochemical and Biophysical Research Communications* **64,** 856–862.

14 Lea, P. J. & Miflin, B. J. (1975) Glutamate synthase in blue–green algae. *Biochemical Society Transactions* **3,** 381–384.

15 Mazelis, M. & Pratt, H. M. (1975) Conversion of 5-oxoproline to glutamic acid in higher plants. *Plant Physiology* **56,** Supplement, p. 7.

https://doi.org/10.23637/ERADOC-1-132

16 MIFLIN, B. J. & LEA, P. J. (1975) Glutamine and asparagine as nitrogen donors for reductant-dependent glutamate synthesis in pea roots. *Biochemical Journal* **149,** 403–409.

17 MIFLIN, B. J. & LEA, P. J. (1975) Glutamate synthase and its role in nitrogen assimilation in plants. *Plant Physiology* **56,** Supplement, p. 88.

18 (NORRIS, R. D.), LEA, P. J. & FOWDEN, L. (1975) tRNA species in the developing grain of *Triticum aestivum*. *Phytochemistry* **14,** 1683–1686.

19 (NORRIS, R. D., WATSON, R.) & FOWDEN, L. (1975) The activation of amino acid analogues by phenylalanyl- and tryosyl-tRNA synthetases from plants. *Phytochemistry* **14,** 393–396.

20 PIERPOINT, W. S. & (DAVIES, R.) (1975) Problems of the reactive species from enzymic and chemical oxidations of *o*-diphenols: Anomalies in the trapping of *o*-quinonoids with benzenesulphinic acid. *Biochemical Society Transactions* **3,** 671.

21 SHEWRY, P. R. & (PETERSON, P. J.) (1974) The uptake and transport of chromium by barley seedlings (*Hordeum vulgare*). *Journal of Experimental Botany* **25,** 785–797.

22 SHEWRY, P. R. & (PETERSON, P. J.) (1975) Calcium and magnesium in plants and soil from a serpentine area on Unst, Shetland. *Journal of Applied Ecology* **12,** 381–391.

23 (WATSON, R.) & FOWDEN, L. (1975) The uptake of phenylalanine and tyrosine by seedling root tips. *Phytochemistry* **14,** 1181–1186.

https://doi.org/10.23637/ERADOC-1-132