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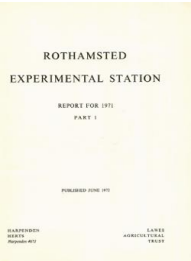

Biochemistry Department

N. W. Pirie

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BIOCHEMISTRY DEPARTMENT

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Everyone is so familiar with the effects of maturity on the quality of fruits and vegetables, and so many aspects of quality can be judged by the unaided senses, that we are apt to forget how little is known about the biochemical basis of changes during maturation. In fruits where, up to a point, the product gets more attractive as it gets older, the disappearance of acids and the appearance of sugars has been studied. Leafy vegetables mature inversely; because they accumulate strongly flavoured materials and fibre, they tend to become less attractive as they mature. Nutritional value may however be increasing at a time when palatability, for people but not for rabbits, is decreasing.

Although our work is not directly connected with the organoleptic aspects of maturity, maturity has a profound effect on many of the processes we study. We have, for example, commented on the effects of leaf age on nucleic acid content, the extractability of ribosomes, the activity and extractability of mitochondria, the ratio of nucleases to phosphatases in so far as it affects ease of purifying these enzymes, and also on the diminished dependence of virus synthesis on illumination in older plants. More recently, emphasis was laid on the importance of harvesting a crop, destined for the bulk production of leaf protein, at the right time because, when the total leaf protein per hectare is increasing rapidly, extractability is diminishing so that the yield of extracted protein may be 20% less than the maximum if harvest is a week too early or late. Age also affects the soluble carbohydrate and non-protein nitrogen content of the leaf, but this is overshadowed by the effects of differences in fertiliser treatment. These differences will be important when full use is made of the various products arising from fodder fractionation. Considerable ingenuity will be needed to assess optimal harvest date when account is taken of the yields and commercial value of fibre, liquor and extracted protein.

Last year we commented on changes in the distribution of diamine oxidase in the roots of clover seedlings as they mature, on the formation of green derivatives of chlorogenic acid in extracts of young but not old tobacco leaves, and on the more complete fixation of nucleic acid derived from tobacco mosaic virus on to fibre from old rather than young tobacco leaves. This year's report comments on the diminution in the ratio of chlorophyll *a* to *b* during maturation of the leaves of some grasses that use the C_4 photosynthetic pathway, and on the greater destruction of β-carotene during the processing of red clover as the crop matures.

These scattered observations have not yet been followed up in sufficient detail to see whether they form the basis for any generalisations; the mechanisms involved have not been satisfactorily unravelled and they do not integrate into a coherent picture of the nature of physiological age. The different aspects of stress, e.g. chronological age, nutritional state, ambient temperature, and illumination, probably affect age-dependent systems differently, but with experimental material as physiologically plastic as a plant, antecedents should be much more carefully specified than they usually are.

Leaf enzymes

Acyl methionine deacylase. Protein chains being synthesised in some organelles of plant cells are thought to start with N-formyl methionine. As the chains grow, the formyl and methionyl residues are removed, possibly sequentially. An enzyme that hydrolyses

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N-formyl methionine was detected in acetone powders of tobacco leaves, and was examined to see if it has a metabolic role in protein synthesis. If it has, its position in the cell would indicate where this type of protein synthesis occurs, and its specificity might explain the occurrence of N-terminal acyl groups in plant proteins.

The deacylase preparations described last year were further purified by chromatography on a cellulose anion exchanger. The enzyme is recovered in excellent yield by a salt gradient and is separated from 90% of the UV absorption of the extract. It is also separated from a peptidase that interferes with measurements.

The resulting extracts hydrolyse N-acetyl arginine, acetyl methionine, acetyl cysteine and formyl methionine in order of decreasing activity. The expected amino acids are released, and, in the hydrolysis of acetyl cysteine, N-monoacetyl cystine is formed. The preparations do not hydrolyse the N-acetyl derivatives of other common amino acids, or, as far as they have been tested, N-formyl derivatives. They do not remove acetyl groups attached to the ϵ-amino groups of either free or histone-bound lysine. They have little or no activity against acylated peptides such as N-formyl methionyl alanine, or N-acetyl glycyl methionine, and although they apparently hydrolyse N-formyl glycyl methionine, much of this activity is due to a contaminating peptidase that removes formyl glycine from the peptide.

Acetyl methionine in excess, but not the other substrates, inhibits hydrolysis. Hydrolysis is also inhibited by comparatively large concentrations (0·5 mM) of p-chloromercuribenzoate. It is not inhibited by iodoacetamide or ethylenediamine tetraacetate, and not stimulated by glutathione or divalent metal ions. The pH optimum depends on the substrate and the buffer used, but is usually between 8 and 9. The molecular weight of the enzyme, judged by its movement down columns of 'Sephadex' is of the order of 150 000.

The enzyme thus resembles the deacylases from other sources, especially that from the seeds of a desert tree, *Parkinsonia aculeata* L. Its properties make it unlikely that its metabolic role is to deacylate the ends of nascent peptide chains. Another enzyme that deformylates peptides is separated from it during purification, and this may well be more relevant to this stage of protein synthesis in plants. Attempts to use the deacylase to detect acetylated amino terminals in proteins have not been encouraging. (Pierpoint)

Tobacco leaf ribonucleases

Purification. Tobacco leaf is a difficult material from which to make purified enzyme preparations because each enzyme accounts for only a small fraction of the total protein. Preparations of ribonuclease (RNase), purified by gel filtration and ion-exchange chromatography, contain several contaminating proteins when examined by polyacrylamide gel electrophoresis and losses during preparation are large. Recent developments in affinity chromatography offer purification procedures giving larger yields of purer enzymes. In addition, the process is quicker. The technique relies on the ability of enzymes to bind specifically and reversibly with ligands that structurally resemble the substrate but are not destroyed by the enzyme. The starting material is chromatographed on a column containing an insoluble matrix to which the enzyme-specific ligand is covalently attached. The desired protein binds to the insolubilised ligand and other proteins pass through unretarded. Bound protein is then released by eluting with a different buffer.

Tobacco RNase is strongly inhibited by purine nucleotides, especially guanosine 2′(3′) mono-phosphate. This, when bonded to 'Sepharose' as the insoluble matrix, binds tobacco RNase strongly and gives a good yield of highly purified RNase. Using this technique, the two tobacco RNases described last year (*Rothamsted Report for 1970*, Part I, 111), were purified to the stage where they appear homogeneous when examined by polyacrylamide gel electrophoresis. The insolubilised guanosine derivatives have also been

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used successfully with RNases from other plants. It is hoped they will prove generally applicable.

Properties of tobacco RNases. Further work on the two RNases confirms their close similarity. Both have molecular weights of about 20 000. The main differences between them are: (1) their pH optima (5·1 and 5·8); (2) their ability to hydrolyse cyclic nucleotides (one hydrolyses only purine cyclic nucleotides, the other will hydrolyse both purine and pyrimidine cyclic nucleotides); (3) their intracellular distribution (one is a soluble enzyme, the other appears to be localised on the ribosomes). The results obtained confirm some earlier work on tobacco RNases and show that the tobacco enzymes resemble those from maize (*Zea mays*). The results contrast with those of Bagi and Farkas (*Phytochemistry* (1967) **6**, 161–169), who found no evidence for the existence of multiple forms of RNases in tobacco. The enzyme they described did not correspond to either of the enzymes discussed above; it had a pH optimum of 6·8 and a molecular weight of 32 000. In addition to the two RNases with molecular weights of about 20 000, tobacco leaf contains two other enzymes which degrade ribonucleic acid (RNA) and have molecular weights of about 50 000 and 31 000. The latter may be the enzyme described by Bagi and Farkas. Little is known about these two enzymes; their activity is small and the larger seems able to degrade DNA in addition to RNA.

Phenol-stability of tobacco RNases. Phenol does not destroy all the RNase activity of crude tobacco leaf extracts, but as the RNases are purified they become more susceptible to denaturation by phenol. This suggests that some component(s) of tobacco leaves protects RNase from phenol. The molecular weight of the component(s) is greater than 60 000. It is not retained by carboxymethyl 'Sephadex' at pH 4·8, indicating that it is acidic. The fraction containing this material prevents the precipitation of RNA by HCl. It is not clear if the same material is responsible for both activities. (Jervis)

Pigments

Chloroplast pigments in plants with the C_4 dicarboxylic acid pathway of photosynthesis. Black and Mayne (*Plant Physiology* (1970) **5**, 738) found that four C_4 plants had a larger ratio of chlorophyll *a* to chlorophyll *b* than plants for the C_3 pathway. Woo, Pyliotis and Downton (*Zeitschrift für Pflanzenphysiologie* (1971) **64**, 400) separated the mesophyll and bundle-sheath chloroplasts of the leaves of several C_4 plants and found that chloroplasts without grana, which occur in the bundle sheath of some C_4 plants, had a greater ratio of chlorophyll *a* to *b* than chloroplasts with grana. This would partly explain the greater ratio for whole leaf, but many C_4 plants have chloroplasts with grana in the bundle sheath. We have long been interested in factors affecting the chlorophyll *a* to *b* ratio in leaves; many C_4 plants were therefore examined to see whether a large *a* to *b* ratio is a characteristic feature, whether or not they contain chloroplasts without grana. Chlorophyll *a* to *b* ratios were determined on a range of C_3 plants as controls. The ratio was determined in aqueous acetone extracts and again after transferring the pigments to diethyl ether.

With members of the Gramineae the average value for C_4 species was about 4·0 and for C_3 species 3·0. The largest values for the C_3 species were as great as the smallest values for the C_4 species. In several C_4 grasses the ratio tended to diminish as the plants aged. Among C_4 Dicotyledons, whole leaves from species of *Amaranthus* (with grana-containing chloroplasts in the bundle sheath) had *a* to *b* ratios as great as 5·6.

A simple method using a top-drive macerator was developed for separating intact bundle-sheath cells from broken mesophyll cells of leaves of C_4 plants. The ratio of fluid to leaf and the blending time were varied depending on the texture of the leaf.

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The effectiveness of the separation was checked by microscopic examination of the residue after blending. The method was satisfactory with all the plants tested, except for mature *Sorghum* leaves, which were too tough, and *Portulaca grandiflora* leaves, which were too soft. The chlorophyll content and the *a* to *b* ratios of the two fractions were determined; the percentage of the total chlorophyll *a* and *b* of the leaf that was present in the bundle sheath fraction was then calculated.

Of 19 species of Gramineae examined, nine had similar ratios of chlorophyll *a* to *b* in the mesophyll and bundle-sheath fractions, and ten had different ratios in the two fractions, with the bundle-sheath fraction having the larger ratio. Table 1 shows some typical results. The ratios can be correlated with the grana configuration of the bundle-sheath chloroplasts where this is known. Because it is not known for all the plants tested, R. H. Turner (Plant Pathology Department) is examining leaf material of several species with the electron microscope.

TABLE 1

Chlorophylls a *and* b *in the mesophyll and bundle-sheath fractions of the leaves of some C_4 plants*

	Age of plants (weeks)	Chlorophyll *a* : *b* ratio			% of total chlorophyll in bundle-sheath		Bundle-sheath chloroplasts
		Whole leaf	Mesophyll	Bundle-sheath	Chl *a*	Chl *b*	
Sorghum, 'Dobbs'	5	4·50	3·81	6·20	40	29	Without grana
Zea mays, Sweet corn	6	4·20	3·80	7·72	19	10	Rudimentary grana
Pennisetum setaceum	11	3·87	3·49	5·17	30	23	Not known
Cynodon dactylon	10	3·37	3·36	3·36	47	47	Grana
Chloris gayana	5	3·42	3·45	3·35	23	24	Grana
Axonopus argentinus	6	3·83	3·79	3·99	20	19	Not known

The distribution of carotenoids between the mesophyll and bundle-sheath was studied in *Zea* and *Sorghum*. The ratio of xanthophyll to carotene is slightly greater in the mesophyll than in the leaf as a whole, and smaller in the bundle-sheath. Consequently a larger proportion of the carotene than of the xanthophyll is present in the bundle-sheath.

Determination of xanthophyll in leaves. The determination of total xanthophyll by standard methods is tedious and a new method involving chromatography on a column of powdered polyethylene is being developed. An aqueous acetone extract of plant tissue is applied to the column and the chromatogram developed with 65% acetone. The xanthophyll pigments are only slightly adsorbed and are eluted from the column ahead of the chlorophylls; the optical density is read between 350 and 550 nm. Extracts from some leaves that brown strongly on grinding contain a pigment, absorbing in the near UV region, that elutes with the xanthophylls. This increases the main xanthophyll absorption peak at about 445 nm. When leaves are ground in aqueous acetone with polyvinylpyrrolidone, the interference is eliminated, but it is more difficult to extract the pigments completely. Chlorophyll breakdown-products, such as pheophorbides and chlorophyllides, in which the phytyl side-chain has been removed, have larger Rfs than chlorophyll on polyethylene and these also interfere. At present the method cannot be used for tissues in which degradation further than pheophytin has occurred. Using extracts from fresh leaves that do not brown strongly, the polyethylene column method gives values for the xanthophyll content up to 10% greater than the standard method; this is not surprising because fewer manipulations are used. (Holden)

Carotenoids in leaf protein. With the leaves of most species, losses of carotene during large-scale pulping and coagulating are small, but with red clover the loss was consider-

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able. In the separated fractions, enzymic breakdown of carotene varied with the species and increased as the leaves aged. Losses were smaller in the pulp and juice than in the fibre. Carotene in the fibre could be stabilised by raising the pH. (Arkcoll and Holden)

Trace elements

The oxidation of pyridoxamine by Mn^{2+} ions. The work on the oxidation of Schiff bases of pyridoxamine with α-oxo acids, reported in 1966, was concluded. Unlike most Mn^{2+} ion-catalysed reactions, no hydrogen peroxide seems to be formed. Ferricytochrome-*c* is reduced by the superoxide anion and has been used as an indicator for this free radical in biological systems. In the oxidation of the pyridoxamine-pyruvate Schiff base, added cytochrome-*c* is reduced immediately before but not after the oxygen uptake commences. This suggests that a free radical, possibly the superoxide anion, initiates the reaction but is unlikely to be formed after the reaction has started.

Distribution of copper and manganese in clover roots. In the work already reported, 0.05 *M* phosphate–0·05 *M* citrate buffer, pH 7, was used for extraction; more copper is extracted into solution at pH 7·5. Frozen clover roots were pulped with buffer and the extract allowed to stand at 2° for several hours before centrifuging at 4000 × *g* for 30 min. About 60% of the total copper and nitrogen and more than 80% of the manganese, diamine oxidase activity and ascorbate oxidase activity, were recovered in the supernatant liquid, which contained little or no cytochrome oxidase. Saturating the extract with ammonium sulphate produced a precipitate which, after dialysis against copper-free distilled water, contained 45% of the copper present in the original extract, 1·6% of the manganese and about 50% of the diamine and ascorbate oxidase activities. More than 75% of the initial manganese remained in the ammonium sulphate solution. Fractionation of the ammonium sulphate precipitate on DEAE cellulose columns yielded small amounts of a manganese-rich fraction, containing 0·05–0·1% Mn, which may be similar to the manganese complexes reported earlier from pea-seedlings (Hill & Mann, *Rothamsted Report for 1963*, 93).

Changes in copper-containing enzymes with changes in copper nutrition of red clover. It was hoped that any genetic differences in the distribution of copper-containing enzymes would be eliminated by using a clone, rather than seedlings, of red clover (*Trifolium pratense*, cv Dorset Marlgrass). No *o*-diphenol oxidase activity was detected in any of the roots although it is abundant in the aerial portions of the plant. Increasing the copper concentration of the nutrient solution from 0 to 0·1, 1·0 and 10·0 μM did not significantly affect the amount of cytochrome oxidase found, but greatly affected the activities of ascorbate oxidase and diamine oxidase. Roots of plants grown without added copper have small amounts of both ascorbate oxidase and diamine oxidase activity indicating that, in spite of stringent purification of the reagents, some contamination with copper still occurred. However, increasing the copper in the nutrient solution from 0 to 0·1, 1·0 and 10·0 μM increased the ascorbate oxidase activity 2, 6 and 6-fold and the diamine oxidase activity 2, 6 and 10-fold. These results suggest that copper, when scarce, is preferentially used by cytochrome oxidase. (Hill)

Environmental influences on free amino acids

Effects of copper nutrition on clover roots. In a new approach to ascertain the role of copper in the metabolism of clover roots, a clone of red clover (*Trifolium pratense* cv Dorset Marlgrass) was grown in aerated nutrient solutions (Long Ashton nitrate type)

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containing 0, 0·1, 1·0 and 10·0 μM copper and the free amino acids were extracted with ethanol from roots approximately two weeks old, i.e. before significant secondary growth or nodulation had occurred. The summation of the total N recovered as amino acids and ammonia per g dry weight was greatest with the least copper and ranged from 100 μg amino N/g dry weight root in plants grown without added copper to 40 μg amino N/g dry weight from plants grown with 10 μM copper. The most abundant amino acids were alanine, γ-amino butyric acid and a fraction tentatively identified as amides. The amides, calculated as asparagine made up about 30% of the total free amino acid N, and did not change significantly with the amount of copper fed to the plant. Increasing the copper supply, greatly decreased alanine and γ-aminobutyric acid, both in their total amounts and in relation to their contribution to the total free amino acid N. Alanine and γ-aminobutyric acid accounted for 36% and 19% respectively of the free amino acid N in plants grown without copper, 19% and 15% in plants supplied with 0·1 μM copper, 13% and 13% in plants supplied with 1 μM copper, and 7% and 8% in plants supplied with 10 μM copper. There is no evidence yet to show whether the changes in amounts of alanine and γ-aminobutyric acid, with increase of copper supply, come from an increase in their further metabolism or a decrease in their formation. (Hill)

The effect of atmospheric composition on *Phaseolus vulgaris*. The effect of different atmospheric oxygen concentrations on the growth and yields of dwarf french beans is described elsewhere (Parkinson & Tregunna, Physics Department). The amino acid contents of 80% ethanol extracts from leaves of these plants were measured in a single-column Technicon automatic analyser to see whether the amounts of serine and glycine, both of which are thought to be involved in photorespiratory pathways, were affected by the atmospheric oxygen concentration during growth.

Both interpretation and calculation of these chromatograms was difficult; of many unidentified ninhydrin-reacting compounds present, some were abundant; some were stable to mild acid hydrolysis. Peak merger between unidentified and known amino acids sometimes prevented measurement of the latter. The larger amounts of glutamine and asparagine in some extracts interfered with serine and threonine determinations: in such extracts these amino acids were determined after mild acid hydrolysis of the extract (1 N HCl at 121°C for 30 minutes).

Of the amino acids that could be measured, serine and glycine were most affected by changes in atmosphere, and their concentration, relative to valine, in leaves receiving only 5% oxygen, decreased to one-half and one-third respectively of the amounts in leaves grown in 21% oxygen. More tyrosine was found in leaves grown in 5% than in 21% oxygen, possibly because it is more readily converted to dihydroxy phenolic compounds at greater oxygen pressure.

The accumulation of amides and the occurrence of β-alanine are often associated with various mineral deficiency states, especially lack of potassium. Both were found in large amounts in most plants, regardless of oxygen concentration; this suggests that, although the solution in which the plants were grown supplied all known nutrient requirements, the plants may be under some stress. (Byers with Tregunna, Physics Department).

Viruses

Infective nucleic acid in leaf extracts. After a decade of intermittent work some definite conclusions are emerging about the factors affecting the infectivity of phenol-treated extracts from tobacco leaves infected with tobacco mosaic virus (TMV), or uninfected

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leaves mixed with TMV. At least three types of effect are involved; they can still be only partially differentiated; hence the delay in bringing the work to a publishable stage.

Fixation on the fibre. Extracts from lower leaves pulped before adding phenol are more infective than extracts pulped with phenol. There is less difference when the interval between pulping and adding phenol is short and no unequivocal difference if air is rigidly excluded during pulping. The normal RNA of infected or uninfected leaves is fixed to much the same extent as TMV:RNA but the nature of the connection and of the oxidative change that prevents fixation, and is itself prevented by the presence of phenol, is not known. Fixation can also be largely prevented by including yeast nucleic acid in the extraction fluid, or by excluding Ca^{2+} from it.

Precipitation and aggregation of RNA in extracts. Several components of leaf extracts, e.g. Ca^{2+}, nicotine and spermine, precipitate TMV:RNA from dilute salt solutions. Even when there is no visible precipitation there is aggregation, so that the TMV:RNA in an extract would be present in fewer infective particles. Salts diminish precipitation but phenol increases it. The interaction of these factors has probably a real but minor effect on ultimate infectivity.

Ribonuclease. Most of the leaf RNase is irreversibly inactivated by phenol and the part that is protected from inactivation is inactive while phenol is present. RNase is therefore probably not an important factor when infectivity is measured soon after making an extract but may become important if there is delay. (Pirie and Bawden)

Reaction of o-quinones with potato virus X (PVX). PVX is difficult to isolate from leaf extracts, and liable, once extracted, to aggregate. A satisfactory method of extracting it into buffered DIECA and minimising its aggregation was devised recently in the Plant Pathology Department: exposed lysine amino groups were found in the virus. This suggests that part of the difficulty in handling the virus may arise from its reaction with enzymically produced *o*-quinones in leaf extracts, and prompted a study of this reaction *in vitro*.

Chlorogenoquinone seems to combine with PVX, modifying its UV spectrum by introducing an alkali-sensitive shoulder between 300 and 320 nm. It also apparently halves the number of amino groups in the virus able to react with 2:4:6 trinitrobenzene sulphonic acid. The modified virus can be centrifuged out of solution, but the dark pellet will not resuspend. Its appearance in the electron microscope suggests that the particles may have been randomly cross-linked into a network rather than aggregated into the more familiar coiled-ropes. (Pierpoint)

Large-scale production of leaf protein

Crops. From most crops, that for other reasons seem useful sources of leaf protein, we now expect to extract 70–85% of the N. This is not all protein N, but the yield of dry extracted protein from Cocksfoot in five or six cuts was 1·9 tonnes/ha, and from S24, S22 and Westerwolds Ryegrasses it was nearly as great. But for the long dry spells last summer, yields would have been still larger for water stress is the main factor limiting the yield of extracted protein. During early spring, Cocksfoot responds to more than 240 kg N/ha/cut, but later in the year 150 to 160 kg seems adequate. The dry spring so minimised leaching losses that a single application of 570 kg N/ha gave a bigger yield than the same amount of N applied in five equal parts during the season.

Mustard and Fodder Radish sown together yielded less than either crop alone, and

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varieties of Spinach, *Amaranth* and *Atriplex*, which had been found hopeful in growth chamber experiments in Sweden, were disappointing. (Arkcoll)

Quality of the extracted protein. Bulk preparations of leaf protein seldom contain more than 10% N; the yields we give recognise this and are not expressed as dry matter but as 100% protein, i.e. N × 6. Lipid accounts for much of the non-protein material, but even after solvent extraction, preparations seldom contain more than 12% N. Batches of protein made by separating the coagulated curd quickly from the liquor, especially when the initial extract is dilute, contain more N. This suggests that contaminating material associates with the protein during processing. We have therefore studied various methods for hastening the separation, hoping thereby to make a better product.

When the coagulated extract flows down a gently sloping perforated screen (holes about 75 μm wide) a curd containing 7–10% dry matter (DM) collects at the foot. This can be pressed to 12–15% DM in a few seconds on a simple cloth belt press and is then dry enough to be fed into the standard belt press with a cloth covering on the perforated pulley. This makes a cake containing 25–30% DM. We hope to organise these operations into a continuous process that will separate most of the liquor from the curd in less than a minute and produce a better quality product. Success depends on handling the curd gently so as not to break the flocks. The same arrangement is not satisfactory for washing the protein.

Galactose, arabinose, xylose and a little glucose are released from protein from Barley, Fat Hen, Fodder Radish, Maize, Red Clover and Rye by heating with 2 *N* H_2SO_4, as reported last year with Cocksfoot, Lucerne, Mustard and Ryegrass. Hence, a uniform group of carbohydrates is probably associated with leaf protein.

The main sugars in the liquor after heat-coagulating leaf extracts are glucose, fructose and sucrose. Sucrose would produce glucose and fructose on acid hydrolysis, but acid extracts from leaf protein contained little glucose and traces of fructose. Both glucose and fructose, however, were found in appreciable quantities in extracts from some cruder laboratory preparations of leaf protein made by coagulating concentrated rather than diluted juice, and also from juice coagulated an hour or more after expression. Fructose in a preparation is probably absorbed from the liquor.

Glucose sometimes occurs in acid extracts from leaf protein without fructose. Fungal amyloglucosidase, which has been used to measure the starch in leaf tissue, was used to digest leaf protein from clover and wheat. These were chosen as samples giving comparatively large and small amounts of glucose on acid hydrolysis. Leaf protein from red clover contained 0·5% and from wheat 0·1% starch respectively—a small proportion of the 5% total carbohydrate determined after acid treatment. The residue from red clover protein after digestion with amyloglucosidase still gave some glucose on acid treatment, indicating the presence of another glucose compound besides starch. (Arkcoll, Davys, Festenstein and Pirie)

Amino acid composition and *in vitro* digestibility. Although there are now many reliable amino acid analyses on unfractionated protein extracted from leaves grown in temperate climates, there are few on protein extracted from leaves grown in the tropics. To reassure overseas workers, and to prevent them doing much unnecessary work, a few samples were analysed and found to have similar compositions to other unfractionated proteins.

Protein was precipitated, by heating to 80°, from fresh and aging extracts of wheat, lucerne and cocksfoot grass. Allowing the extract to stand before heating, or leaving the heated extract at room temperature for up to 3 hours, made no difference to either the total N in the precipitated protein or to the amount of N released by *in vitro* digestion for 24 hours with thioglycollic acid-activated papain. However, it is not known if the

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rate of digestion, or the rate of release of individual amino acids, differs; both will be studied using these preparations. Similar results were obtained with protein precipitated from extracts stored overnight at + 4° before heating, but protein made from extracts kept overnight at room temperature contained less N and were less digestible. Because of co-precipitation, protein made by adding acid to a fresh extract usually contained less N than the corresponding heat-precipitated preparation, but its *in vitro* digestibility was sometimes greater.

Lucerne extracts were fractionated by controlled heating, and by centrifuging, as described last year. The N contents of the cytoplasmic fractions, especially those obtained by acid precipitation from the 55° supernatant, are less than is usual in comparable fractions made from other species, confirming the results obtained at the Central Food Technological Research Institute, Mysore. These chloroplast-free preparations, some of which contain only 10% N, are about 95% digestible *in vitro* (using activated papain); this supports the view that the association of chloroplastic protein with other material is responsible for unfractionated proteins being less digestible. (Byers)

Wet preservation of leaf protein at room temperature. Washed and acidified (pH 4) cake keeps for only 3 or 4 days at room temperature because of the growth of *Mucor racemosus*. This fungus has adapted itself to the process and is difficult to avoid. The acidity prevents anaerobic bacteria from putrifying the protein and the large water-holding capacity limits fast aerobic growth unless free surface moisture is present. Thus *M. racemosus* is favoured except on unwashed lucerne in which the residual liquor seems to contain an inhibitor.

Fortunately *M. racemosus* is so sensitive to acetic acid that 0·5% (cake weight) usually inhibits it completely. Slower-growing fungi begin to appear after a few months but can be controlled by stronger acetic acid. There are small differences in the keeping quality of protein from different species, presumably because of inhibitors or variations in the final pH reached during washing. Storage for 6 months required 1% acetic acid for lucerne and 3% for wheat. Five per cent salt is enough to prevent *M. racemosus* growing but 15% is needed to avoid some other fungi appearing after a week. (Arkcoll)

Machinery. The auger feed (20 cm diameter) to the large pulper, that was mentioned last year, works well. It pushes the crop to within 1 or 2 cm of the beaters and, by preventing even momentary blockage, ensures even loading; power consumption is thus diminished 25%. An auger necessitates pre-chopping the crop. An 'Atlas Model C' chaff-cutter, installed between the elevator and the auger, works well on most crops but is not yet perfectly adapted for gripping soft grasses. The new feed arrangement removes the hazard of large lumps of crop suddenly entering the pulper. The fluid coupling was therefore removed from the belt drive to the machine. This not only saves a small power loss, but also allows us to permute our pulleys and extend the speed range at both ends. (Davys and Pirie)

Cooperation with other organisations. More protein was sent to the University of Ife because, in preliminary trials at the University Teaching Hospital, children with kwashiorkor recovered with a rapidity that greatly excited the medical staff. Protein was also sent to the A.R.C. Poultry Research Centre (Edinburgh) for test on colostomised birds which should supplement the earlier trials made at the Rowett Research Institute and by Messrs Bibby, and was sent for trials on fish to Dr. Hickling and the N.E.R.C. Fisheries Biochemical Research Unit in Aberdeen. Twenty samples of protein processed in different ways were sent to the Rowett and the Department of Biochemistry in Aberdeen for animal feeding comparisons designed to find out which stages in normal

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processing may be harmful. A small sample of protein labelled with ^{15}N was made for the National Institute for Research in Dairying (N.I.R.D.).

On behalf of the Department of Agriculture at the University of Reading, 8 tons of kale was processed; they wish to investigate the increased efficiency with which the fibre residue can be dried and the nutritive value of the product. There have been several meetings to discuss these matters with members of the staffs of N.I.R.D., the Grassland Research Station, the Rowett Research Institute, the National Institute for Agricultural Engineering and with Messrs. Dengie Crop Driers (Southminster, Essex). Cooperation on the use of the liquor as a microbial substrate continues with the National College of Food Technology.

As a result of the meeting in Coimbatore last year, to which they sent a representative, we have had active cooperation from Messrs. Alfa Laval, who have already established a company, in cooperation with organisations in Denmark and Hungary, to exploit leaf protein. Alfa Laval lent us two designs of 'decanter' centrifuge, hoping that they would separate the coagulated protein curd satisfactorily. We are promised the loan of a more suitable model next year.

Dr. D. Chaturvedi (India) and Mr. J. Mwara (Kenya) came for 1 or 2 weeks to learn methods and Professor O. A. M. Lewis (Department of Botany, Durban) came for a longer period because he is hoping to establish leaf protein production in the Transkei. Several commercial concerns in Britain and elsewhere have shown interest and the Instituut voor Bewaring en Verwerking van Landbouprodukten (Wageningen) has bought an IBP extraction unit.

Staff

At the expense of Messrs. Lysoform, M. N. G. Davys attended a conference on the use of lucerne, and products derived from it, in California and visited Lysoform in Brazil. N. W. Pirie was sent by the Inter-University Council to discuss the possibility of starting work on leaf protein in Guyana, and also took part in symposia on 'Food Science and Nutrition in the Seventies' in Texas, on 'Research and Education in Fundamental Biology Relevant to Human Welfare' in Seattle, and on 'Research and Education in Developing Countries' in Caracas.

R. N. S. Olsson resigned.

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