ROTHAMSTED RESEARCH

Report for 1972 - Part 1

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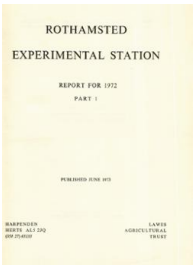

Biochemistry Department

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BIOCHEMISTRY DEPARTMENT

N. W. PIRIE

An independent Biochemistry Department was constituted in 1947 out of the Section of Biochemistry, that had been part of the Plant Pathology Department for the preceding seven years, and the ARC Unit of Soil Biochemistry. Work on the extraction and state of oxidation of soil manganese, which had been started in the Unit, continued until 1949. Because it seemed likely that much soil organic matter was composed of the more resistant parts of fungal mycelia, we studied the N compounds in several species; that work continued until 1957. The Department did not then completely lose interest in events below ground; work on nematode hatching factors started in 1955 and continued until 1967. After a five-year interval interest in soil has reawakened because of the striking beneficial effects of the 'whey', that is a by-product of leaf protein production, on soil structure.

The work of the original Section was all connected in some way with virus research, and this connection has continued to dominate our programme. At first we were mainly concerned with the chemical and physical properties of plant viruses and of some other proteins peculiar to infected leaves. Then we studied the reactions that proceed in leaf pulps and that affect viruses, or infective nucleic acid made from them. Changes in pH first attracted our attention, later it was the action of ribonuclease, of substances released by mitochondria, of phenolic compounds and quinones, and of nucleic acid precipitants such as calcium and spermine. Work on the amount of virus in an infected leaf is bedevilled by uncertainty about the completeness with which virus is being extracted from the tissue. We compared several methods of mechanical disintegration and introduced enzymic methods depending on carbohydrases from snails and moulds.

Virus synthesis is an aspect of leaf metabolism. It is greatly affected by the nutritional state of the leaf; TMV-infected tobacco, stunted by lack of N but given ample P and K, is an extreme example, 80% of the protein-N can be in the TMV. Shading increases the susceptibility of plants to infection, and illumination increases the rate of multiplication of virus once infection is established. In spite of prolonged effort we could not associate these differences with clearly defined differences in the concentration of various organic acids in the leaf or with activity of various enzymes associated with photosynthesis. A change in leaf colour is often an early effect of infection. Yellowing could result either from decreased synthesis or increased destruction of chlorophyll. We chose to study the latter, first by studying the conditions in which chlorophyllase is active, and later lipoxidase. Chlorophyllase splits off phytol, leaving chlorophyllide which is still green; processes associated with lipoxidase action decolourise chlorophyllide. Many points of great biochemical interest arise from this work—but the basic question remains unanswered.

During the 1950s, improvements in the design of centrifuges and electron microscopes facilitated biochemical work on organelles. While studying the usual contaminants of virus preparations, and the increase in leaf respiration soon after infection, we made the first reasonably clean preparations of leaf ribosomes and mitochondria. The enzyme activities associated with them were studied in some detail.

Studies on enzymes are the backbone of biochemistry. We have worked with about 20 besides those already mentioned. Amine oxidase was pursued most persistently because of controversy about the presence of copper and pyridoxal in it and also the possibility that it is involved in alkaloid synthesis and that it controls the level of indoleacetic acid

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in plants. Because thiaminase occurs in bracken, but not in higher plants, the enzyme was purified and specific inhibitors were looked for in the hope that they could be used to kill bracken. As with viruses, there is uncertainty about the completeness with which enzymes are released from tissues by the usual methods of disintegration: what seems to be increased content may simply be increased accessibility. The activation or release of latent enzyme was demonstrated with phenolase and cytochrome oxidase; we are aware that changes in accessibility may complicate our attempts to correlate differences in the activity of copper-containing enzymes with differences in the amounts of copper supplied to plants.

The research described so far, with the possible exception of that on bracken and the nematode hatching factor was not designed to be useful in immediate practice. The nutritional factors influencing blackening in potatoes were examined briefly, but we did not add much to existing knowledge. Our main attempt to be practically useful is the work on the extraction of leaf protein—from 1940 to 1948 on the laboratory scale only, and since then in large-scale equipment that we have designed and steadily improved. This work illustrates a defect in the 'customer : contractor' principle. Until about five years ago the work had no 'customer', attempts were indeed made to stop it. The cogency of the various points made in 1950 was first recognised abroad—recognition has now spread to Britain and there is no longer any need for large-scale work at Rothamsted. We can concentrate on our proper job of studying the agronomic problems involved in this unrivalled method of producing protein.

The Biochemistry Department has always been one of the smallest in Rothamsted. At first it was also the most cramped and this led many of our colleagues to seek more ample accommodation. Two are now professors, one is head of a department in Scotland and one is head of a CSIRO Division in Australia. There is now ample space in the Department and several staff vacancies. Accommodating the new interests of the incoming head may therefore be possible by complementation rather than transformation.

Studies originating from virus research

Potato virus X (PVX) modified by coupling with *o*-quinones. When PVX is exposed to enzymically oxidising chlorogenic acid, and re-isolated, some of its properties are changed. Its UV spectrum is modified and centrifuged pellets are coloured; its tryptophan fluorescence is diminished and a longer wave fluorescence introduced. It also produces less colour with 2 : 4 : 6 trinitrobenzene sulphonic acid (TNBS) and moves faster towards the anode on electrophoresis, suggesting that some of viral ϵ-amino groups have reacted with chlorogenoquinone. However, the modified virus is still highly infective towards *Chenopodium amaranticolor* and Xanthi tobacco. Because there is some evidence that this modification by quinones occurs naturally in senescing infected leaves, the properties of the modified virus may well be relevant to the survival of PVX in the field and its transference to new hosts.

Modified but highly infective forms of PVX can be prepared with other reagents that react with the ϵ-amino groups of proteins. Thus reaction of PVX with pyridoxal phosphate, followed by reduction of the Schiff's base produced, gives a product whose absorption and fluorescence suggests that it contains about 1 pyridoxal phosphate per protein sub-unit. The amount of unreacted virus in the preparations is much too small ($< 5\%$) to account for their infectivity. Similarly PVX preparations in which, on average, one or two lysine groups have been modified with TNBS, are highly infective. However, more extensive substitution of the 11 lysine groups in each protein sub-unit destroys infectivity without grossly affecting the virus particles; some of the ϵ-amino groups of PVX are therefore essential for initiating infection. (Pierpoint)

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Fixation of ribonucleic acid by tobacco-leaf pulp. The precise conditions of extraction affect the amount of virus nucleic acid appearing in phenol-containing extracts from TMV infected tobacco leaves. The extraction of normal nucleic acid from uninfected leaves is also affected (*Rothamsted Report for 1971*, Part 1, 125). Most of the phenomena observed with virus-infected leaves can be replicated with pulp from uninfected leaves to which yeast nucleic acid is added. This artificial procedure is useful in preliminary analysis of the system because it permits more uniform subdivision of smaller batches of leaf than was possible with the methods used hitherto. Fixation is increased by an increase in pH or Ca^{2+} concentration, and diminished by 30 minute autolysis, especially in alkaline conditions, and by Ca chelators. As with infected leaves, fixation increases with leaf maturity and, although Ca^{2+} is an essential component of the system, when present in physiological concentrations, it does not act in the absence of other leaf components. (Pirie)

Purification of ribonucleases. The affinity chromatographic method developed for purification of tobacco-leaf ribonuclease (RNase), (*Rothamsted Report for 1971*, Part 1), was used to recover RNases from the 'whey' from leaf protein production. RNases are of commercial interest and no cheap large-scale source is yet available. Conventional methods of enzyme isolation and purification would make recovery of RNases from this dilute source uneconomic but a method based on specific affinity could yield large amounts of enzyme relatively cheaply.

The affinity adsorbents used for tobacco RNase purification were prepared by covalently attaching a guanosine monophosphate (GMP) derivative to a porous matrix of agarose gel. But the gel beads are soft and their distortion on prolonged use stops the flow. Porous glass beads are a more rigid supporting matrix for affinity adsorbents intended for large-scale use.

Porous glass beads have been used for molecular sieving, expecially of viruses, but their use is limited because they bind protein molecules by ion-exchange. This behaviour would be undesirable in affinity chromatography and it can largely be overcome by reacting surface silanol groups on the glass beads with trialkoxysilanes. By using an amino-alkoxysilane, surface ion-exchange groups can be blocked and at the same time, amine groups are introduced that can be used for covalent attachment of enzyme inhibitors. Guanosine 2′(3′) phosphate is a potent inhibitor of tobacco RNase and some other relatively purine-specific RNases. When covalently attached to porous glass beads, GMP will reversibly bind RNases from tobacco, lucerne and a fungal RNase (RNase T_1 from *Aspergillus oryzae*). Bound enzyme can be released by washing the adsorbents with a very dilute solution of GMP ($10^{-6}M$). Enzymes purified in this way appear to be homogeneous when subjected to polyacrylamide gel electrophoresis. The porous glass adsorbents are stable and do not lose their excellent flow properties during prolonged use. The columns used so far hold only 1–2 ml of adsorbent but they will bind all the RNase from several litres of leaf extract. (Jervis)

Studies connected with trace elements

Changes in free amino acids in clover roots with changes in copper supply. The large amounts of alanine, γ-amino butyric acid and amides in copper deficient clover roots (*Trifolium pratense* L. cv. Dorset Marlgrass), reported last year, were, in part, the consequence of inadequate aeration. Clover roots grown in continuously aerated and non-aerated nutrient solutions (Long Ashton, nitrate type) containing 0, 0·1, 1 and 10 μM Cu^{2+}, were harvested when about two weeks old and the free amino acids extracted with 80% ethanol. The total soluble amino acid N was greatest in non-aerated roots without Cu and decreased with increase of Cu; in aerated roots this soluble amino acid N changed

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little with the three lowest amounts of Cu but doubled with the greatest amount. The most important components were alanine, asparagine, and γ-aminobutyric acid and were greatest in the copper deficient non-aerated roots. Ethanolamine increased with increase of Cu in non-aerated roots but changed little with increase of Cu in aerated roots. Arginine and histidine formed less than 3% of the soluble N in non-aerated roots. In aerated roots without Cu, arginine and histidine accounted for more than a quarter of the soluble amino acid N but decreased in roots supplied with more Cu. Acid hydrolysis of the ethanol extracts did not increase the amount of glutamate but always increased the amount of aspartate, confirming that the amides were mostly asparagine. Small amounts of β-alanine always occurred in extracts from non-aerated roots and the amounts found were not affected by Cu supply; acid hydrolysis of these extracts did not increase the amount of β-alanine. No free β-alanine was found in extracts from aerated roots although small amounts were found after acid hydrolysis

Subcellular distribution of some copper-containing enzymes in clover roots. The 10^2 *g* precipitate centrifuged from red clover root extracts in 0·4*M* sucrose, contained 50%, and the supernatant after centrifuging at 10^4 *g* 44%, of the total ascorbate oxidase activity; this suggests that as in other plants ascorbate oxidase is present in two forms, one bound to cell walls and the other soluble. The supernatant fraction contained over 80% of the diamine oxidase activity with the remainder in the 10^2 *g* precipitate. No *o*-diphenol oxidase activity was found in any fraction. (Hill)

Indole acetic acid oxidase inhibitors and peroxidase in cotton plants grown with different amounts of calcium, phosphorus, and manganese. Le Mare (Chemistry Department, p. 51) has shown that increasing the phosphate supply to cotton plants (*Gossypium hirsutum* L.) grown with small amounts of Ca, increases the Mn in the leaves. Cotton plants were grown in nutrient solutions in a controlled environment chamber with 0·15 and 2·25 *mM* Ca, 0·065 and 1·00 *mM* P, and 0·009, 0·082, 0·155 and 0·300 *mM* Mn.

Peroxidase activity was greatest in the leaves of plants grown with the smallest amounts of both Ca and P and decreased with increase in the amount of Mn. Peroxidase and Mn^{2+} ions with trace amounts of a suitable phenol catalyse the aerobic oxidation of indole acetic acid (IAA). All cotton leaf extracts had peroxidase; although some of the leaves had visible deposits of MnO_2, no significant change in oxygen uptake was found on adding IAA. These leaf extracts inhibited the oxidation of IAA by horseradish peroxidase with Mn^{2+} and p-chlorophenol; the weight of leaf required to give a 50% decrease of the oxygen uptake varied linearly with the logarithm of the Ca/Mn ratio in the nutrient solution and was increased by increasing the phosphate. (Hill, with Le Mare, Chemistry Department)

Selective ligand (affinity) chromatography. Unsuccessful attempts were made to prepare chromatographic materials for the selective adsorption of metalloproteins by covalently bonding chelating agents to cellulose, sepharose, porous glass and silica gel. However, these supports covalently bonded with salicylic acid, salicylaldoxime, or 8-hydroxyquinoline, chelate strongly with ions of transition elements. All complexes were strongly coloured but those with cellulose and sepharose were unstable and colour was continuously leached, even by distilled water. This suggests that other sepharose derivatives, now frequently used for the affinity chromatography of some enzymes, may not be as stable as is often claimed. 8-Hydroxyquinoline and other chelating agents can be coupled, using alkoxy silanes, to the silanol groups (Si–OH) of porous glass to give adsorbents which are more stable to acid and neutral solutions than complexes prepared from cellulose and

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sepharose. Porous glasses are expensive and ground glasses have insufficient area to give absorbents with a large capacity. Silica gels have not hitherto been used to prepare adsorbents for affinity chromatography; they have good flow properties, a large number of reactive silanol groups and are cheap. Silica gel coupled with 8-hydroxyquinoline will, within the limits of detection, remove traces of Cu^{2+}, Zn^{2+}, Mn^{2+} and other transition element ions from $2M$ $Ca(NO_3)_2$, $2M$ KNO_3, $0{\cdot}75M$ $MgSO_4$, and $1{\cdot}3M$ NaH_2PO_4. Adsorbed Cu^{2+}, Zn^{2+}, and Mn^{2+} are easily removed by acid; Mn^{2+} elutes at about pH 3, Zn^{2+} at pH 2, and Cu^{2+} at pH 1. This silica gel complex is stable, at least for several months, in neutral, acid and many organic solvents but decomposes in aqueous solutions above pH 9. A phenylhydrazine derivative has also been prepared from silica gel for the adsorption of aldehydes and many other functional groups can also be covalently bonded to give complexes with selective adsorbent properties. (Hill)

Studies connected with the bulk production of leaf protein

Determination of sulphur in leaf proteins. The oxygen combustion method (Schöniger (1955) *Mikrochimica Acta* 123, and (1956) 869) for determining S in organic compounds is preferable to the more widely used wet oxidation methods because methionine-S (met-S) is fully recoverable from both plant material and compounds containing much methionine. Sulphate is determined by measuring spectrophotometrically the amount of chloranilic acid released from Ba chloroanilate in acid solution (Bertolacini & Barney (1957) *Analytical Chemistry* **29**, 281).

Relation of total S to S-containing amino acids in leaf proteins. Methionine is the limiting amino acid in leaf protein; when added to diets containing the unfractionated product, nutritive value improves. On the other hand chloroplast-free preparations, with a similar methionine content, have a biological value approaching that of casein, indicating that lack of availability of methionine, rather than its absolute amount in the unfractionated product, is responsible for the apparent deficiency.

To assess potential nutritive value, the total-S containing amino acid content is used more often than the content of methionine alone. With leaf proteins this presents certain difficulties; methionine can be reliably measured but the published figures for cystine show wide variation, irrespective of whether it is determined directly or as cysteic acid. This is partly due to the small amount of cyst(e)ine in relation to the other amino acids (1–2%) and partly to destruction by non-protein material in the sample.

Two series of preparations were selected: both unfractionated and fractionated protein made from three age cuts of lupin, and parallel preparations made from three separate batches of lucerne. The ratio of S : N in all preparations ranged from 0·063 to 0·072 and 0·059 to 0·069 for lucerne and lupin respectively. Met-S, calculated from the methionine content obtained by amino acid analysis, accounted for 39–45% of the total S. If all the S not accounted for by methionine is cyst(e)ine, there would be 2·3 to 2·7% of it in the protein (N × 6·0) in these preparations. The only cystine figures derived directly from amino acid analysis which are at all comparable are those from chloroplast-free preparations, confirming the earlier remarks. Because of the presence of lipids in extracted leaf proteins containing chloroplastic material (from 20–40% of dry matter) samples from a lucerne chloroplastic fraction were extracted with various hot solvents. The ratio of S to N and calculated cystine (as percentage of protein content) were not affected.

Because other insoluble S compounds may be present, calculating the cystine-S content of a protein by deducting the met-S from the total-S should not be regarded as a substitute for determining cystine, wherever this is possible, but it is a useful method for establishing an upper limit.

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From these results it would appear that leaf proteins may contain more cystine than has usually been accepted. However, the problems associated with the nutritional unavailability of both cystine and methionine when chloroplastic material is present remain, although the preparations themselves are not deficient in total-S containing substances. (Byers)

Chlorophyll breakdown products in leaf protein preparations. Almost all the green pigment in freshly expressed juices of the leaves of most crop plants is chlorophylls *a* and *b*. A trace of pheophytin *a* but not of pheophytin *b* is usually present because the slight acidity of the juice removes Mg from chlorophyll *a* though not from chlorophyll *b*. When juice is kept at 20°C for 24 hours the amount of pheophytin *a* increases and a trace of pheophytin *b* is formed. Pheophytins may then account for up to one-third of the total 'green' pigment. On storage at 2°C for several days the only apparent change is a slight increase in the amount of pheophytin *a*.

Leaf protein preparations (LP) contain up to ten easily recognisable pigments. Heat-coagulated, unwashed protein contains chlorophylls *a* and *b* and small amounts of the isomers *a'* and *b'* that are formed by heat treatment. Nearly all preparations contain pheophytin *a* but some have none because it is converted into pheophorbide *a* by chlorophyllase action; pheophytin *b* is rarely present. When the leaf is rich in chlorophyllase (e.g. wheat, lucerne) the chlorophylls may be converted into chlorophyllides by removal of the phytyl side-chain. This is clearly seen in LPs coagulated at 80° although not in those coagulated near 100°C. Heat is needed for chlorophyllase to act in an aqueous system and chlorophyllide is not formed when juice is kept for 24 hours at 20°C or for several days at 2°C.

Acid-washed LPs contain little or no chlorophyll *a* but some chlorophyll *b* usually remains. From leaves with low chlorophyllase activity (e.g. potato, kale) about 80% of the total pigment is pheophytins *a* and *b*, but pheophorbides may also be present. With high chlorophyllase activity about 90% of the total extractable pigment is pheophorbides *a* and *b*.

Freeze-dried preparations have the same pigments as fresh ones; further breakdown products may be formed on storage depending on the conditions. During storage at — 20°C or at room temperature in a metal foil pack there is little change, but protein stored in polythene packs loses some coloured degradation products and several extra pigments are found. The loss of extractable pigment may be due to bleaching or to pigments becoming more tightly bound to the protein. Some of the additional pigments seem to be 'changed' pigments (Bacon & Holden (1967) *Phytochemistry* **6**, 193) derived from the pheophytins and possibly also from the pheophorbides. A cocksfoot protein stored in polythene for two years had eight red-fluorescing bands on a TLC plate in addition to the pheophytins and pheophorbides. The 'changed' pigments are oxidation products and their presence is an indication of unsatisfactory storage conditions.

Xanthophylls in leaf protein preparations. When juice from a range of species is stored for 24 hours at 20°C or for several days at 0°C, xanthophyll is more stable than carotene, but during heat coagulation of the protein the loss of xanthophyll is greater than that of carotene. LPs therefore have smaller xanthophyll to carotene ratios than the juices from which they were made.

The total xanthophyll fraction from a fresh juice has an absorption curve with peaks at 446 and 473 nm and a shoulder at 420 nm. Individual xanthophylls were separated by chromatography on columns of cellulose and other adsorbents. Lutein (a dihydroxyxanthophyll), accounts for about half of the total xanthophyll; violaxanthin and neoxanthin, which are both 5,6 epoxy pigments, account for the rest, except for about 5% of minor

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pigments that may be formed during chromatography. Acidification of juice to pH 4 alters the shape of the absorption curve and the absorbance is diminished by about 20% at 446 nm.

Freshly made unwashed LPs contain only a little violaxanthin; it is mostly converted into luteoxanthin (with one furanoid and one epoxy group) but some is further converted into auroxanthin (with two furanoid groups). There is little change in the lutein and neoxanthin.

The xanthophyll fraction from acid-washed LPs has an absorption curve similar to that given by an acidified juice. Although maximal absorption is usually at about 450 nm it is sometimes at about 420 nm. A trace of violaxanthin is still detectable and there is usually more auroxanthin than luteoxanthin. Neoxanthin is decreased; treatment with acid is known to convert it into neochrome, a 5,8 epoxide, and this may occur during the making of LP. Treatment with acid converts lutein into two *cis*-isomers and deoxylutein (Livingston, Nelson & Kohler (1969) *Journal of the Association of Official Analytical Chemists*, **52**, 617) the relative amounts of the pigments being dependent on the concentration of acid. It seemed possible that this might happen during the acid washing of LP but there is little evidence for this.

Freeze-dried preparations stored with air present lose xanthophyll fairly rapidly. Violaxanthin, neoxanthin and their breakdown products disappear, leaving lutein accounting for more than 80% of the xanthophyll fraction. The lutein is more stable than the carotene so the xanthophyll to carotene ratio is much greater in stored preparations than in fresh. (Holden)

The relationship between protein and pigments in leaves. Freshly made LPs all contain from 0·7 to 1·7 mg β-carotene/g dry weight suggesting a close relationship between the protein and pigment contents of the preparations. Chlorophyll, xanthophyll and β-carotene were determined in the juices of a range of crop plants throughout the growing season. Good correlations between the pigment values and the protein contents of the juices were found, especially within species. Fractionating leaves with the IBP unit gives a good measure of moisture and fibre content. These correlations could now be combined to estimate simultaneously the moisture, protein, pigments and digestibility of fresh fodder crops by measuring the volume of extractable juice and either β-carotene or total xanthophyll. (Arkcoll and Holden)

Carbohydrates in leaf protein preparations. Protein preparations from wheat and red clover, containing 5% and 7·5% carbohydrate respectively, as determined after hydrolysis by heating with $2N$ H_2SO_4, were incubated with Pronase at 37°C and pH 7·2. About one-third of the carbohydrate from wheat, and half of that from red clover, was released into the enzyme digests, compared with that released by acid hydrolysis. The reducing sugar content of the enzyme digests was small and no free sugars or oligosaccharides could be detected by thin-layer chromatography. Heating the enzyme digests in $2N$ H_2SO_4, or incubating them with pectinase at pH 4·5, increased the reducing sugar content and galactose, arabinose and glucose were then detected.

Incubating protein with pectinase at pH 4·5 released one-third of the acid-released carbohydrate from wheat and one-quarter from red clover, but the proportion of reducing sugar increased during incubation to a large fraction of the total. Snail digestive juice at pH 4·5 released a little more carbohydrate from wheat protein and a little less from red clover, than did pectinase, but the same sugars were detected. The amount of carbohydrate released by pectinase was not affected by prior incubation of the protein with Pronase.

The residues after successive incubations with buffer, Pronase and pectinase contained

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one-quarter of the original carbohydrate for wheat and one-tenth for red clover, the only sugar detected being galactose, Extraction of the protein preparations with 2 : 1 chloroform : methanol, before the incubation sequence, decreased the final residual carbohydrate by one-quarter.

Some carbohydrate released by Pronase, is evidently bound initially in a protein–polysaccharide complex which is not accessible to pectinase. Glycoproteins consisting of arabinan-galactan bound to protein have been found in the primary walls of plant cells and some of them have been extracted with acid as water-soluble complexes from leaves. (Festenstein)

Effects of leaf protein 'whey' on soil. When added to water-logged Saxmundham soil, with an inherently poor structure, 'whey' promotes the growth of *Clostridium butyricum* and *C. pasteurianum* (see Skinner, Soil Microbiology Department Report, p. 88). The gas produced may increase soil volume threefold; after drying it is still doubled because the pores are stabilised by microbial polysaccharide (with P. Bullock, Pedology Department). The beneficial effect persists if the polysaccharide is not leached away. If 'whey' is being returned to bare soil, the area should not be much smaller than that from which the crop came or germination may be inhibited, but it can be used more concentrated on established lucerne. During the growth and fermentation of clostridia, N is fixed and there is some diminution of nematode infestation.

Agronomy. The yield of extracted protein is increased when cocksfoot grass is given 160, instead of 80, kg N/ha for each of four or five cuts, although the yield of dry matter is not increased. The effect of larger applications, e.g. 240 and 640 kg of N, in the form of 'Nitro-Chalk', depends on the weather. When plants are growing well in a warm dry spring the N can be assimilated, but much is lost by leaching in a wet spring, and such heavy applications may cause damage when growth is slower later in the year. With some irrigation in summer, Westerwolds and R.V.P. ryegrass yielded more than 1·8 tons of dry extracted protein per hectare. Irrigation had the added advantage of producing autumn grass of spring quality; unirrigated autumn grass gives a protein coagulum that is difficult to separate by the methods we use.

Protein quality. Differences in trypsin digestibility, and nutritional value not accounted for by the small differences in amino acid composition, of different leaf protein preparations have been known for many years. In collaboration with the Rowett Research Institute and the Department of Agricultural Biochemistry in the University of Aberdeen the effects of variations in processing conditions are being studied and tend to confirm our opinion that protein is damaged by conjugation with other leaf components. Protein coagulated from lucerne extract diluted with two or three times its volume of water is better than protein coagulated from undiluted extract. If the diluent water is added during the initial pulping, and in the form of a second extract, it also increases the amount of protein extracted. The addition of sulphite, to inhibit the oxidation of phenolic compounds, improves quality still further. (Arkcoll)

Machinery and cooperation with other organisations. Cooperation with Messrs. Alfa Laval, commented on last year, was intensified. Drs A. Moberg and J. Wihlborg were with us for several weeks testing a 'PX' centrifuge from which the sediment can be discharged intermittently without stopping the machine. The difficulties encountered were all matters of adjustment that can be remedied, and this centrifuge makes it easy to separate colourless 'chloroplast' protein from green 'cytoplasmic' should that be thought desirable. But the unit tested could take only 3–9 litres/minute and is a formidable

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machine. If 3 tons of crop were being processed per hour, there would be 4–10 times as much extract as that.

Because of the desirability of separating coagulum from 'whey' quickly and simply, we continued our attempts to remove most of the 'whey' by allowing the curdled extract to flow down a perforated slope. We are confident that this technique could be made to work, but we are not yet satisfied with our design.

The National Institute for Research in Dairying (Shinfield) has installed a 3 tons an hour pulper and belt press. Their primary interest is in the fibre residue because of the ease and economy (*Rothamsted Report for 1970*, Part 1, 118) with which it can be dried as cattle fodder. The unfractionated extract is fed to pigs. We visited NIRD regularly during the summer to help with installation and with the first few runs.

In cooperation with ADAS the digestibility of many fibre samples by rumen contents was measured. Some showed the slight diminution to be expected because of the removal of soluble carbohydrate, but others were improved. This suggests that the removal of substances that inhibit rumen digestion can sometimes compensate for the removal of carbohydrate.

The 'whey' can be most simply disposed of by using it as irrigation water, thus making use of its N, P, K content and the soil-improving properties already commented on. We have often advocated its use as a microbial substrate. In cooperation with the Process Technology and Chemistry Divisions at the Atomic Energy Research Establishment (Harwell), the 'whey' has been usefully concentrated by reverse osmosis. Work on this will continue and will be extended to 'whey' made during the mechanical dehydration of potatoes. (Arkcoll, Davys, Festenstein and Pirie)

Staff changes, visitors and travel

After 14 years, during most of which he was supported by the Rockefeller and Wolfson Foundations, M. N. G. Davys had to resign. L. Jervis also resigned. I. Aarek (Nutrition Institute, Oslo University), R. Kamali (Rowett Research Institute, Aberdeen), M. de Romanett (Oregon) and T. P. Yorks (Texas A & M University, College Station, Texas) came to study various aspects of leaf-protein production. G. I. Karis (Department of Food Science, University of Reading) came to learn methods of carotenoid analysis.

On behalf of 'Find Your Feet Ltd', Davys went to India to advise on establishing a leaf-protein extraction unit in Coimbatore. Pirie visited Calcutta to advise on the progress of leaf-protein work in the Indian Statistical Institute; Mexico under the Royal Society scheme of exchange visits; Seattle for the General Assembly of the International Biological Program; and Norway to lecture on leaf protein and related subjects to Messrs. Collett A/S, the Nutrition, Food Science and Technology Group of the Norwegian Chemical Society, the Botanical Institute of the Agricultural College, and the Intermediate Technology Group.

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