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ROTHAMSTED RESEARCH

Rothamsted Report for 1967

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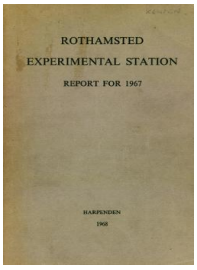

Biochemistry Department

N. W. Pirie

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BIOCHEMISTRY DEPARTMENT

N. W. PIRIE

Leaf metabolism predominates over metabolism in other tissues when plants are growing most actively, and studies connected with leaves continue to predominate in our programme. The partial or complete disappearance of chlorophyll is an early sign of the impending degeneration of a leaf from senescence, malnutrition or infection. We still do not know whether it is the consequence of chlorophyll being destroyed faster or of its synthesis slowing, but our knowledge of the enzyme systems involved is now approaching the stage at which this question can be studied. Leaf proteins—for example, enzymes, viruses and potential foods—readily form complexes with quinones, unsaturated fatty acids and other leaf components, especially when extracts are even briefly exposed to atmospheric oxidation. As a result, enzyme activity, infectivity or digestibility may be diminished and false conclusions may be drawn about the activity of these proteins *in vivo* and about their potentialities. We are studying the nature of these complexes and the steps that can be taken to prevent their formation. Many enzymes contain, as essential components, those metals needed in traces for plant growth. The obvious explanation for the specific symptoms of a "trace-element deficiency" is that the deficient plant cannot make enough of some enzymes; the symptoms are the metabolic consequences of this. We have begun a quantitative study of the distribution of some trace-elements among different tissue fractions to see to what extent a balance sheet can be drawn up showing all the forms in which certain trace-elements occur. Progress is being made in the study of the factors, enzymic and otherwise, that influence the susceptibility of plants to infection by viruses and the subsequent multiplication of viruses in them. Leaf protein is made in bulk to satisfy requests from developing countries where its use as a food is being studied. We are now mainly concerned with the value of the by-products, leaf residue and the uncoagulable components of the extract, and with the yields possible with different systems of husbandry. We have made a pulper and press that, working on 2–3-kg samples of leaf, simulate closely the action of large-scale extraction equipment. With this unit, meaningful agronomy on replicated plots can now start.

Enzyme actions

Chlorophyll destruction. Preliminary experiments with Sephadex G-100 suggested that chlorophyllase extracted from sugar-beet (*Beta vulgaris*) leaves had a molecular weight of about 37000. It is now clear that there are several chlorophyllase fractions with a range of molecular weights. It seems that the enzyme tends to aggregate either with other proteins or with itself. Thus when a crude extract is concentrated by ultrafiltration much

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of the chlorophyllase activity emerges with the high-molecular-weight fractions on analysis by gel-filtration, and the low-molecular-weight chlorophyllase peak is broadened because of aggregation or association. Aggregation is, at least in part, reversed by treatment with acetone, and low-molecular-weight chlorophyllase with enhanced specific activity can be made in this way. There is no evidence that this enzyme occurs *in vivo* in multiple forms, as some other enzymes do.

A new method of purification was devised which omits acetone precipitation and overcomes some of the problems caused by aggregation. The crude extract at pH 7 is passed through a carboxymethyl Sephadex ion-exchange column, which retains most of the low-molecular-weight chlorophyllase. Neither the high-molecular-weight chlorophyllase nor most of the other proteins are absorbed. The enzyme is eluted by increasing the salt concentration. The eluate is concentrated by ultrafiltration and chromatographed by gel-filtration on Sephadex G-100. At this stage the enzyme is still stable. It is purified further by a second adsorption, after ultrafiltration, on carboxymethyl Sephadex and elution with a linear salt gradient at pH 5·5. The chlorophyllase is now, weight for weight, approximately 400 times as active as the starting material, but is less stable. It is stabilised by bovine serum albumin, but not by EDTA, mercaptoethanol, cysteine or freezing. Some protection is given by 50% glycerol. Analysis by electrophoresis shows that the enzyme is not yet pure, and that the isoelectric point is near pH 7.

The preparations described above are made from aqueous extracts of the acetone-powder of sugar-beet leaves, further extracted with ethanol and diethyl ether. When fresh leaves were ground directly with aqueous buffer solution and the extract centrifuged most of the chlorophyllase activity was in the supernatant fluid, and little in the chloroplast fraction. Gel-filtration showed that much of the chlorophyllase was in the high-molecular-weight fraction. Traces of chlorophylls in the supernatant fluid were slowly converted into chlorophyllides, even though free from acetone. A non-aqueous method of chloroplast preparation would probably be necessary if the chlorophyllase is to be retained in the chloroplast fraction and this used as a method of purification. The chloroplasts of sugar beet are unusually fragile. To locate the enzyme, a species is being sought that is as rich in chlorophyllase as sugar beet but with more robust chloroplasts. A "clean", stable, enzyme preparation is needed for work on the nature of the reactions that lead finally to colourless products; this objective has nearly been achieved. (Bacon and Holden)

Leaf lipoxidase. As part of an investigation on chlorophyll breakdown in whole leaves and fractions separated from them, the enzyme lipoxidase is being studied. This enzyme is one factor in a system that will bleach chlorophyll *in vitro*, but it is not known whether it is involved in its degradation *in vivo*. Lipoxidase in leaves is associated with the plastid fraction, from which it can be partially extracted with detergents such as Triton X-100, but the residue contains nearly as much activity as appeared to be in the plastids originally. The detergent is not completely removed from enzyme preparations by various treatments such as dialysis, gel filtration

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on Sephadex G-100, or by precipitation of the protein with ammonium sulphate, but lipoxidase determinations are not affected by the residual traces. (Holden)

Reactions of quinones with other components of leaf extracts. The ortho-quinones formed by the enzymic oxidation of the polyphenols of leaf extracts react readily with many leaf proteins, modifying their chemical and physical properties. In this way they inactivate both leaf enzymes and viruses. Not enough is known about this reaction to predict which proteins are susceptible, what the properties of the modified proteins are and how the reactions can best be prevented. Earlier studies of the quinones derived from chlorogenic and caffeic acids have therefore been extended to cover their reactions with amino acids, peptides and proteins.

Both quinones react readily with the α-amino groups of amino acids, especially in alkaline solution, giving highly coloured compounds. These undergo secondary rearrangements, often involving the absorption of oxygen, and produce brown products similar in appearance to the polymerised products formed from the quinones alone. The red-purple compounds initially formed from proline are stable enough to be characterised spectrophotometrically, but not stable enough to be isolated. The compounds formed initially from other amino acids are less stable; the absorption spectra of the compounds they rearrange to, as well as the amount of oxygen absorbed during the rearrangement, depend to some extent on the amino acid. Acetylation of the α-NH_2 of amino acids keeps them from reacting with quinones, unless, like cysteine or lysine, they have other reactive groups. The SH- of cysteine is very reactive and seems to have a greater affinity for quinones than most amino groups.

The reaction of the quinones with several di-, tri- and tetra-peptides suggests that the main course of the reaction, and the colour of the products, are determined mainly by the amino acid with the terminal NH_2. Thus glycyl-alanine reacts like glycine, and leucyl-glycine like leucine. However, there are differences, especially in the secondary reactions. More oxygen is absorbed in the prolonged reaction of glycyl-glycine and quinones, for instance, than in that of glycine, and the products absorb less light at 700 mμ. Similarly, although β-alanyl-L-histidine reacts initially more like β-alanine than L-histidine, the final colour of the solution resembles that produced when histidine reacts with quinones.

The reaction of the *o*-quinone derived from chlorogenic acid with purified serum albumin was studied as a model for the reaction of quinones with leaf proteins. At least two types of modified albumin are formed. The protein produced by small amounts of quinone is colourless and is formed without any oxygen uptake. It is not produced from albumin pretreated with such SH-reagents as *p*-chloromercuribenzoic acid, and so is likely to be an albumin-*S*-quinone complex. A red compound formed in the presence of a 2–3 fold excess of quinone probably contains quinones attached to groups of the albumin in addition to -SH. Both these modified albumins can be separated from unreacted albumin by chromatographing them on ion-exchange media near the isoelectric pH of albumin. Their U.V. spectra differ only slightly from that of albumin, and

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preliminary analysis of the red product suggests that only about seven of its 600 or so amino acids have reacted with quinone. (Pierpoint)

The oxidation of *o*-aminophenol by leaf polyphenoloxidase. The phenoxazone structure occurs naturally in various antibiotics and biological pigments. It is thought to arise from the enzymic oxidation of *o*-aminophenols to *o*-quinonimines followed by the condensation of these compounds with more *o*-aminophenol. Some tropical plants contain a flavoprotein that specifically catalyses the synthesis of isophenoxazine from *o*-aminophenol, but the similarity of this reaction to the oxidation of *o*-dihydroxyphenols raises the possibility that it could also be catalysed by the more widespread polyphenoloxidase.

Polyphenoloxidase preparations from tobacco (*Nicotiana tabacum*) leaves oxidise *o*-aminophenol to isophenoxazine. The reaction is slow compared to the oxidation of caffeic acid, and the enzyme has a very small affinity for *o*-aminophenol. That the reaction is catalysed by the polyphenoloxidase of the preparations is suggested by its susceptibility to substances that inhibit this enzyme but not flavoproteins, and by the relative constancy of the ratio of the two oxidative activities in different leaf extracts: there is no suggestion that a flavoprotein enzyme is involved. *o*-Aminophenol oxidation is speeded eight times by adding traces of *o*-dihydroxyphenols, suggesting that it can also be brought about non-enzymically by *o*-quinones produced from the enzymic oxidation of *o*-dihydroxyphenols.

These oxidations are clearly relevant to the synthesis of phenoxazones by polyphenoloxidase-containing fungi, but it is difficult to judge whether they have any physiological significance for higher plants, and the matter will not be pursued. (Pierpoint)

Chloroplast inhibitors. Two reagents that prevent the browning of leaf extracts, potassium ethyl xanthate (PEX) and benzene sulphinic acid (BSA), were shown (*Rothamsted Report* for 1965, p. 110) to do so by reacting with quinones produced from the oxidation of leaf phenols. These compounds may also react with plastoquinone (PQ), which is present in chloroplasts where it is involved in oxidation–reduction reactions. Such a combination with BSA or PEX may inhibit the normal reactions in which PQ is involved. The effect of PEX and BSA on the reduction of phenol-indo-2:6-dichloro-phenol (PIDP) by extracted chloroplasts was tested, as this reaction involves PQ.

BSA inhibits the reduction of PIDP by chloroplasts extracted from *Nicotiana tabacum*. A little evidence suggests that it does this by other means that by combining with PQ. By contrast, PEX stimulates this reaction, possibly by uncoupling the reduction from the rate-limiting phosphorylation. PEX, also, seems not to combine with PQ.

Virus multiplication. Viruses affect the activity of various enzymes in infected leaves. Reciprocally, chemical and physiological changes affecting the activity of enzymes can be expected to control the rate, or even the possibility, of virus multiplication. So many samples must be assayed to

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measure multiplication rates satisfactorily that it seems necessary to have a chemical method, and for use on leaf discs (weighing about 50 mg) it should not call for more than 0·2 mg of tobacco mosaic virus and it should not be influenced by other leaf components that may vary in concentration during the course of infection. A method meeting these requirements has not yet been fully developed, but progress is being made with one that depends on the absorption of light at 260 mμ after differential precipitation with ammonium sulphate. Using this method in its incompletely developed state, virus can be detected in two days, and it shows increased multiplication when discs, floating on half-strength Vickery nutrient solution, are illuminated with 320 ft candles rather than 50. (Olsson)

Distribution of metalloproteins in plants. To find how much metal is necessarily supplied to a growing plant by the seed, the copper contents of seeds of some cereals and legumes were determined using sodium diethyldithiocarbamate as the colorimetric reagent. After washing with *N*-HCl to remove surface contaminants containing heavy metals, the copper contents ranged from 6 to 20 ppm, the smaller seeds tended to give the larger values, but contained less copper per seed. Thus a single clover (*Trifolium pratense*) or lucerne (*Medicago sativa*) seed contains 3–4 ng, whereas the cereals contain 170–350 and green gram (*Phaseolus radiatus*) contains 1000. Unwashed seeds contain about 40% more.

Clover seeds, after surface sterilisation with concentrated sulphuric acid, were germinated and grown in water culture. The aerial parts of seedlings three weeks old were harvested and freeze dried; this freeze-dried material had a copper content of 200 ppm. It was disrupted with an all-glass homogeniser using a hexane–carbon tetrachloride mixture of density 1·32 and, after filtering, the extract was centrifuged to separate the sub-cellular components. Most of the copper (about 80 ppm) was in the fraction sedimented by centrifuging at 1000 × *g* for 30 minutes; a green turbid fraction, presumably containing the chloroplasts, which floated after centrifuging at 50000 × *g* for 2½ hours, contained 18 ppm copper and the lower paler green clear fluid 14 ppm. The actual weights of copper in these three fractions are in the ratios 3 : 1 : 1·8. The activity of the copper-containing enzymes in these subcellular fractions is being measured.

Distribution of diamine oxidase. Large amounts of diamine oxidase occur in the stem apices of most legumes. An enzyme system oxidising putrescine to Δ′-pyrroline has now been found in stem apices of tobacco (*Nicotiana tabacum* c.v. White Burley) and honeysuckle (*Lonicera periclymenum*). Whether this system consists of a single component, as in legumes, is not known, but this observation supports the suggestion that, in some plants at least, oxidation of tryptamine by an amine-oxidising system may be involved in forming auxin. Because diamine oxidase contains copper, its quantitative distribution will be studied, but other work on it has stopped. (Hill)

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Leaf protein and its by-products

Equipment. Basically, the large-scale pulper and belt-press remain unchanged, but some new alloy steels were tried for the beater tips. "B A 500" wears well and withstands the flints occasionally present in the crop. Juice coming from the press carries with it 1–2% of the fibre. During long runs there was a "bottle-neck" at the stage where this fibre was strained off. We therefore made a rotating-drum filter from which this fibre is continuously back-washed, and it and the wash-fluid are used for the second extraction of the fibre residue. Apart from minor and correctable faults it works satisfactorily.

Experience with the laboratory-scale press (*Rothamsted Report* for 1966, p. 105) showed up the deficiencies of domestic mincers for small-scale disintegration. After trying many variations of speed, end plate, knives, and even after going to the extreme of driving both the scroll and the knives independently, we abandoned the principle of the slow-speed mincer and made a smaller version of the large-scale pulper. It takes 0·5 kg of leaf per minute and produces a pulp closely resembling the one made by the large machine. It has a variable-speed drive, and, because it is intended for use in various co-operative projects of the International Biological Programme, is so designed that it can be mounted on the platform of a Land-Rover and run from the power take-off. (Davys and Pirie)

Extraction technique. During large-scale extractions only a few seconds elapse between pulping and pressing; in laboratory-scale work, with present equipment, the interval is necessarily longer. Many latex-containing leaves are rich in protease, and even with wheat there is significant proteolysis in the extract in a few hours. We have long suspected that this is not the only way in which coagulable protein can be lost, but that the amount of protein in the extract diminishes when it is left in contact with pulped leaf fibre. Experiments with red clover (*Trifolium pratense*), rye (*Secale cereale*), kale (*Brassica oleracea*) and mustard (*Sinapis alba*) justified this suspicion and, with the last, showed that the seriousness of the loss in coagulable protein when the pulp was left for 2 hours before pressing increased steadily with the age of the crop. (Street)

Even when leaves are disintegrated thoroughly, and when the fibre is separated from the extract within a few minutes, the fibre from some species may retain more than half of the total nitrogen of the leaf. This retention can often be plausibly explained by the acidity of the leaf, or by the presence in it of tannins and polyphenols, but these explanations do not cover all species that extract badly. These leaves may contain some other type of protein complex, or the unextracted nitrogen may not occur as protein. Only 50–75% of the nitrogen of red clover is usually extractable. More than half of the residual nitrogen becomes soluble when the fibre is incubated with papain activated by KCN, but most of it is still precipitable by trichloroacetic acid. Similarly, 90% of the nitrogen is soluble in a mixture of phenol, acetic acid and water, but after recovery from the solvent it is only partly digested by papain. During these treatments pectin is also extracted from the leaf residue. There is some evidence that poor

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extraction, and poor digestibility by papain, are consequences of complex formation between protein and pectin. (Ahmad)

Digestibility of isolated protein. Leaf protein, like soya-bean and some other plant proteins, is more slowly digested *in vitro* than casein, but may have comparable nutritive value. There are two obvious explanations for this anomaly. Casein is soluble, and so exposes more points of attack than an insoluble powder; this may make less difference to digestion when churned in the gut. Complexes, with lipids for example, may be more readily attacked by the complex mixture of enzymes encountered *in vivo* than by the simpler systems used *in vitro*. Using papain activated by thioglycollic acid, the effects of complex formation and its reversal were studied. The rate of *in vitro* hydrolysis decreased when leaf protein, containing 7–10% moisture and the normal 25–30% of lipid, was heated for several hours at 100° C in air or in nitrogen. Hydrolysis was unaffected by heating dry samples, and samples extracted with lipid solvents can be heated for several days without a significant loss in digestibility. The poor *in vitro* digestibility of briefly heated samples can be restored by solvent extraction. Some solvent-extraction procedures lessen the digestibility even of fresh leaf-protein preparations. After extraction with 2 : 1 chloroform : methanol containing 1% HCl both the *in vitro* and *in vivo* digestibility of leaf proteins decreases, probably because such proteins are very hard, horny and difficult to wet.

When the acid-solvent extracted protein is washed in several large volumes of water before drying the *in vitro* digestibility is restored to slightly above that of protein before lipid extraction.

Experiments with rats at the National Institute for Research in Dairying showed that true digestibility *in vivo* followed the same trends as the above *in vitro* results using papain. However, with these samples several *in vitro* pepsin digestion procedures gave poor correlation with *in vivo* true digestibilities in rats. (Buchanan)

Yields, new species and by-products. Partly because of increasing skill in handling the extraction machinery, and partly because of successful timing of harvesting and resowing, the yield of extracted protein from a hectare within a year has now reached 1·4 tons. This was got in each of three experiments in which winter wheat was cut twice and then followed either by two crops of radish, three of mustard or one of kale. The wheat yielded 690 kg/ha, whereas, on a comparable plot, two cuts of unvernalised rye yielded 785 kg/ha. With still more skill in organising a succession of crops we expect to be able to reach 2 tons/ha. Red clover, in spite of an attack of Wilt and of the poor extraction already commented on, yielded 1247 kg/ha. This would be an excellent crop to use in the temperate zone if extraction could be improved and the by-products fully used.

Of several plants newly tested, only *Tetragonia tetragonoides* (New Zealand Spinach) grew fast and extracted well. Rejected plants were *Galega officinalis*, *Ricinus communis*, *Nicandra physaloides*, *Lathyrus tingitanus*, *Impatiens roylei* and *Cardiospermum halicacabum*.

For as long as leaf protein was being made on an experimental scale

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only, it was reasonable to discard both the fibre residue and the liquor that carries away soluble but uncoagulated material from the protein. On all suitable occasions we have pointed out that this policy would be neither hygienically possible nor economically desirable once continuous production started. A series of analyses of the fibre (Byers & Sturrock, *J. Sci. Fd Agric.* (1965), **16**, 341) showed that the nitrogen content ranged from 0·7 to 3·3%, with most values lying between 1·5 and 2·5. The fibre is probably more digestible than usual forage containing a similar percentage of nitrogen, because the crop providing it would be younger. Plans are therefore being made for tests elsewhere of its feeding value—perhaps with urea added.

Research institutes and manufacturers have, from time to time, tried samples of the liquor as substrates for microbial growth. These tests were not followed up, though one firm said it was worth ten shillings a ton, which is more than the cost of the electricity used in pulping enough crop to make a ton of liquor. The composition depends greatly on the species of plant and its maturity when cut. Thus, during this year, the dry matter of the liquor has ranged from 20 to 47 g/l, the nitrogen content from 0·4 to 1·5 g/l and the carbohydrate from 4 to 40 g/l. Some preliminary trials, in co-operation with the Soil Microbiology Department, show that about half the nitrogen in red-clover liquor could be used by a yeast. The analytical work done on ryegrass is fully described in the report of the Chemistry Department, p. 50. (Arkcoll and Byers)

Co-operation with other institutes. With grants from the International Biological Programme, Dr. R. N. Joshi (Department of Botany, Marathwada University, Aurangabad, India), Miss P. G. Lidman (Institute of Plant Physiology, Lund, Sweden) and Dr. G. Kamalanathan (Home Science College, Coimbatore, India) came to learn the use of the laboratory-scale extraction equipment, because it is hoped that this equipment will become standard in the various centres where leaf protein is being made. A similar visit by a scientist from Nigeria is arranged, and co-operation with New Guinea and Uganda is being discussed. Dr. Kamalanathan gave us valuable advice on techniques of presentation suitable for use in South India. A food-manufacturing company (Nordreco AB. Bjuv, Sweden) invited Ahmad to spend most of the summer in their works studying the extraction of protein from pea haulms on a commercial scale; he concluded that 350–400 kg/ha could be produced as a by-product. Our results here suggest a figure nearer 600. During 1967 supplies of protein were sent to 18 institutes in the United Kingdom or overseas for demonstration or experiment. The two largest consignments were 414 kg (wet weight) of ryegrass (*Lolium perenne*) protein to Dr. A. C. Field (Moredun Institute) for an experiment with sheep and 38 kg (freeze-dried) of red-clover protein to Dr. C. Gopalan (Nutrition Research Laboratory, Hyderabad, India) for human trials. We are co-operating with the Agricultural Institute, Castleknock (Ireland), over the installation there of a "village unit". My initial guess that it would take 25 years for the idea of using leaf protein to be accepted seems to have been about right. (Pirie)

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Self-heating of hay

Work on the self-heating of moist hay, to temperatures greater than those reached through the action of thermophilic micro-organisms, is now ending. It started as a study of "farmer's lung disease" and was continued because various points of biochemical interest arose. Methods for avoiding dangerous self-heating are now well known, and the phenomenon in hay seems not to differ significantly from spontaneous combustion in other organic materials.

Hay self-heated above 70° C lost more pentosan than cellulose. The unheated hay contained only small amounts of soluble polymers of glucose and pentose, and these increased on self-heating to 70° C; those of pentose more than glucose. On self-heating to 100° C the glucose polymers hardly increased further, but the pentose polymers increased appreciably, especially oligosaccharide pentose. Xylose was the chief hydrolysis product detected chromatographically. Free pentose increased along with the soluble pentose polymers, but not when the hay self-heated to 168° C; this hay contained very little free sugar. Moistened hay heated at 80° C in 2-lb Kilner jars with access of air changed like self-heated hay, becoming acid and losing cellulose and pentosan, particularly pentosan.

The first change in the carbohydrates of hay is the hydrolysis by plant enzymes of non-reducing sugar (fructosan and sucrose) to reducing sugar. When hay is incubated with water at 37° C there is appreciable hydrolysis of non-reducing sugars; in a few hours with some samples and a few days with all. All hays hydrolysed added sucrose, but there was no apparent correlation between the rate of hydrolysis and the amount of soluble carbohydrate initially present in the hay. The enzyme is only partly soluble. The residue after extracting all soluble carbohydrate still had enzyme activity. Fresh grass, after repeated maceration, also retained enzyme activity in the residual fibre. Some of the enzyme may be adsorbed on the fibre, or may be a component of the cell wall. (Festenstein)

Potato cyst-nematode (*Heterodera rostochiensis*)

Hatching factor. Work on the hatching factor since the Report for 1966 has proceeded in collaboration with Professor A. W. Johnson's team at Nottingham University. It was agreed that the initial task was to simplify and improve the purification procedures before starting further chemical investigation. Some of the required improvements were already in hand, others were begun after the start of collaboration.

Potato plants were re-examined as a source of the hatching factor. The plant material was extracted with ethyl acetate, and the hatches obtained with the various extracts compared. Roots were a better source of the hatching factor than leaves and stems, or tubers. The hatches obtained with equal weights of the ethyl acetate extracts were in the ratio 16–64:4:1. Roots from young plants (3–4 weeks after planting) gave more hatching factor (>64 fold) than the roots from older plants (12 weeks after planting). Tests showed that root diffusate from young actively

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growing plants remains the best source of hatching factor, but young roots seem likely to be a better source during the winter.

Chromatographic fractionation on a silica column has been substituted for ethyl acetate extraction in the initial steps of purification; it produced a similar purification (90% less in weight while retaining 85% of the activity), but is faster and more convenient.

Some preliminary investigations of the behaviour of the hatching factor on thin-layer chromatograms were mentioned earlier (*Rothamsted Report* for 1965, p. 113). A detailed study of various thin-layer media and solvent systems established the most suitable conditions. The hatching factor was located at defined R_F values and was removed for testing, or transferred for further chromatography with little loss of activity. The active fraction recovered from the chromatograms was colourless and weakly fluorescent. Further tests are in progress to establish thin-layer chromatograms as a routine method for detecting the hatching factor. The conditions established for thin-layer chromatography were applied to large-scale columns for use in the final stages of the purification.

The cyst and egg walls. The cyst wall is 72% protein, and 18 amino acids were obtained on acid hydrolysis. The eggshells contain chitin (*Rothamsted Report* for 1966, p. 108), but not the cyst walls, which contain small amounts of lipid (2%), carbohydrate (0·5%) and inorganic matter (ash 5%). The dark pigments of the cyst wall are probably not indole-containing melanins because characteristic degradation products (dihydroxyindoles and pyrrole-carboxylic acids) were not obtained after alkali fusion or permanganate oxidation of insoluble material from acid hydrolysis. Polyphenols (2%) were detected in the acid hydrolysates.

The tough, resistant qualities of the cyst wall can thus probably be attributed to the tanned protein formed by the reaction of polyphenols with the protein of the female cuticle. (Clarke)

Staff and visiting workers

P. J. G. Mann retired after a period of ill-health. He came to Rothamsted in 1940 as part of an Agricultural Research Council Unit on soil biochemistry. In 1946 that Unit was disbanded and he joined the Biochemistry Section, which became a Department in 1947. For a short time he continued to work on soil, and problems connected with it, but soon turned his attention to plant enzymes—in particular peroxidase, in which he had been interested since 1930, and diamine oxidase. In a series of ten papers he patiently and skilfully unravelled some of the confusion about its metal content and its relations with pyridoxal.

N. W. Pirie attended the "International Symposium on Protein Foods and Concentrates" held in the Central Food Technological Research Institute (Mysore). J. Bodden was awarded the B.Sc. degree of London University.

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