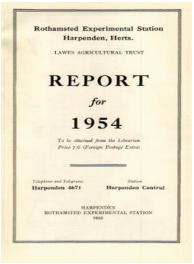
ROTHAMSTED RESEARCH

Report for 1954

Full Table of Content



Biochemistry Department

N. W. Pirie

N. W. Pirie (1955) *Biochemistry Department ;* Report For 1954, pp 73 - 79 **- DOI: https://doi.org/10.23637/ERADOC-1-76**

BIOCHEMISTRY DEPARTMENT

N. W. PIRIE

Margaret Holden has been seconded to the West African Cocoa Research Institute, to study the composition and metabolism of normal cocoa leaves and leaves from plants with Swollen Shoot.

W. R. Smithies resigned and W. S. Pierpoint joined the department.

N. W. Pirie attended a symposium on " Solar Energy and Wind Power " organized by UNESCO and the Indian Government in New Delhi in October.

THE INFLUENCE OF LIGHT ON THE RESISTANCE OF FRENCH-BEAN LEAVES TO INFECTION WITH TOBACCO NECROSIS VIRUS

(G. H. Wiltshire)

The study of the changes in concentration of organic acids in French-bean leaves has been extended to malic and citric acids. The latter, at about 0·02*M* concentration, is known to reduce infectivity of TNV when mixed with the virus *in vitro*.

Several methods for the extraction and estimation of these acids have been tried. Approximate results could be obtained by analysis of an ether extract of heat-dried leaf, but heat converted some of the malic to malonic and acetic acids, which were not present in the unheated leaf. The procedure eventually adopted includes aqueous extraction of leaves previously ground to a powder while frozen at —20°, purification of the extract on ion-exchange resins and partition chromatography of the acids on silica gel. Acids titrated in successive fractions of the eluate were identified by chromatography on paper.

Under normal greenhouse conditions in June, leaves contained about 3·9 m.equiv. of malic and 2·6 m.equiv. of citric acid per 100 g. fresh weight. In October the values were 2·9 and 2·6 respectively. The total organic acid content (of which malic and citric acids make up about 70 per cent) fell by 17 per cent in plants placed in the dark for 3 days in June. Nearly half the malic acid was lost, while the citric increased by about 15 per cent.

Leaves detached from the plant 2 days before inoculation and floated in water produced less lesions than leaves left on the plant; the reduction may be attributed to dilution of the inoculum with water. Otherwise these leaves behaved usually like attached leaves, producing more lesions if kept in the dark before inoculation, though sometimes there was no increase. Leaves floated in 0·003*M* solutions of malate or citrate, then washed before inoculation, produced fewer lesions than those in water but more than those in 0·03*M* malate or citrate. Potassium malonate had the same effect as malate and citrate. It is not yet known whether the infiltrated acids accumulate or are metabolized by the leaf. Since infiltration with these acids decreased lesion number equally in the light and in the dark, it is unlikely that resistance to infection depends simply on the concentration of any one of them.

https://doi.org/10.23637/ERADOC-1-76

A decrease in resistance to infection similar to that produced by darkening was found in plants kept in the light but deprived of carbon dioxide. Both treatments prevent photosynthesis, and may thus make the leaf more easily infected in the same way.

Plant Enzymes Catalysing Oxidations

(R. H. Kenten)

This year, work has been started on the enzymatic constitution of the bracken plant, and shortly, studies of the effect of certain mineral deficiencies on the enzymes of plants will be undertaken. It is hoped that these researches may lead to the formulation of a bracken-killing agent on the one hand and the development of biochemical tests for mineral deficiencies on the other. The work involves the demonstration and estimation of enzyme activities of plants. Unknown factors which affect the activities of the plant enzymes during their extraction or assay can vitiate such studies. It is for this reason that a study of broad-bean-leaf polyphenol oxidase has been undertaken. By analogy with the known phenol oxidases it is most likely that the broad-bean enzyme is a Cu-protein.

Polyphenol oxidase

Water extracts of broad-bean leaves when assayed at pH 6–7, in the usual way, for polyphenol oxidase often show little or no activity. Prolonged incubation (48 hours) of such extracts at 37° (in the presence of toluene) leads to an increase in activity. When the extract is incubated in the presence of certain proteolytic enzymes (e.g. trypsin) large increases in activity are obtained in a short time (1–2 hours), but if incubation is prolonged (48 hours) the activity is largely destroyed. Large increases in the polyphenol oxidase activity of the leaf extracts can be brought about instantaneously by treatment with certain anionic wetting agents (e.g., sodium dodecyl sulphate) or by acidification to pH 4. Inactive leaf extracts show no increase in activity after dialysis for 48 hours at 0°, but the treatments described above bring about large increases in the activity of dialysed extracts. The nature of these phenomena is not yet clear, but, since all of the treatments which result in an increase of activity can also lead to protein denaturation or degradation, the hypothesis is advanced that at the normal pH of the sap (about pH 6) the polyphenol oxidase is largely present in the form of a complex with another protein or large peptide which prevents it exerting its normal catalytic function. Whether such complexes are present *in vivo* or are formed by protein interaction during the preparation of the leaf extract is not known. A knowledge of these phenomena, apart from its importance in plant-enzyme studies, may help in the elucidation of the activation and inactivation processes which sometimes accompany the extraction and purification of plant viruses.

Enzymes of bracken

Studies have been made of the peroxidase, catalase and polyphenol oxidase activities of the bracken plant at various stages of growth. With the exception of peroxidase, which may be absent in the mature leaf, all three enzymes were found in the rhizome,

https://doi.org/10.23637/ERADOC-1-76

stem and leaf throughout the year. Large differences were found in the catalase and polyphenol oxidase activities of the different plant parts, and in the leaf these activities varied greatly with age. The growing portions of the plant (frond bud, young leaf, rhizome tip) had the highest polyphenol oxidase activities. For example, the activities of the frond bud or young leaf were some 60–300 times greater than that of the rhizome and about 20 times that of old leaf. On the other hand, while the growing portions had a higher catalase activity than that of the rhizome, the highest activity was found in the fully expanded leaf. Peroxidase activity was very low in all of the samples examined.

Manganese Oxidation by Illuminated Chloroplast Preparations

(R. H. Kenten and P. J. G. Mann)

It has been suggested that a manganese oxidation–reduction cycle may be concerned in the reactions of photosynthesis. We have previously found, in preliminary experiments, that added Mn^{++} is oxidized by illuminated chloroplast preparations. This reaction has been further investigated in view of its possible physiological significance.

Two methods have been used to follow the oxidation. At high concentrations of Mn^{++} the oxidation product can be estimated manometrically by measuring the N_2 output on addition of hydrazine. At physiological concentrations of Mn^{++} the manometric method is not sufficiently sensitive, and a spectrophotometric method has been evolved which is suitable for estimating Mn^{+++} at a concentration of 2–7 μg./ml. Using these methods, it has been shown that the oxidation proceeds at physiological concentrations of Mn^{++} and depends on the intensity of illumination and the amount of chlorophyll present in the chloroplast preparations. Oxidation proceeds to about the same extent in both pyrophosphate and orthophosphate buffers. In pyrophosphate the oxidation product accumulates as soluble manganipyrophosphate, but in orthophosphate the oxidation product is insoluble. Like MnO_2, it can be dissolved by treatment with pyrophosphate containing Mn^{++} to give manganipyrophosphate, but it differs from hydrated MnO_2 in that it reacts much more slowly with hydrazine in the manometric method of estimation, though finally giving a greater total N_2 output. It is of interest that we obtained a similar oxidation product in previous work in which the oxidation of Mn^{++} was brought about by coupling enzyme systems producing H_2O_2 with peroxidase systems in orthophosphate media.

The mechanism of the oxidation of Mn^{++} by illuminated chloroplast preparations has not been completely elucidated. A thermolabile factor is involved, added catalase partially inhibits the oxidation, while added peroxidase increases the rate of oxidation, though the amount required to produce a significant increase is large in comparison with that present in the chloroplast preparations. These results, in conjunction with our previous results on the oxidation of Mn^{++} by peroxidase systems, suggest that, at least in part, the oxidation is due to peroxidase systems.

https://doi.org/10.23637/ERADOC-1-76

Plant Amine Oxidase and Some Reactions it Catalyses

(P. J. G. Mann)

The study of the products formed as a result of the oxidation of putrescine, cadaverine, lysine and *o*-amino-β-phenylethylamine catalysed by plant amine oxidase has now been completed, and the work has been prepared for publication. These oxidations all produce heterocyclic compounds and, when putrescine and cadaverine are the substrates, the products are key substances in model syntheses of pyrrolidine and piperidine alkaloids. The results therefore support the suggestion that the enzyme may be concerned in the synthesis of alkaloids. From reaction mixtures with DL-lysine as substrate, following catalytic hydrogenation, the 2 : 4-dinitrophenyl derivative of DL-pipecolinic acid has been isolated. The fact that the enzyme catalyses the oxidation of both L- and D-lysine, previously suggested from observations with L- and DL-lysine as substrate, has been established thanks to a gift of D-lysine from Dr. J. P. Greenstein. From reaction mixtures with cadaverine as substrate *iso*tripiperideine, a polymer of Δ′-piperideine, has been isolated. One of the main products of the hydrogenation of *iso*-tripiperideine is 2 : 3-dipiperidine. The latter compound is a derivative of the alkaloid anabasine, which is isomeric with nicotine. Indole has been isolated after the enzyme-catalysed oxidation of *o*-amino-β-phenylethylamine in 90 per cent yield. During the course of this work it has been shown that peroxidase catalyses the oxidation of indole by hydrogen peroxide.

In some plants tryptamine can function as a precursor of 3-indolylacetic acid. We have previously shown that the oxidation of tryptamine is catalysed by plant amine oxidase, and have suggested that the reaction product would be 3-indolylacetaldehyde, which, on further oxidation, catalysed by aldehyde oxidase, would give 3-indolylacetic acid. Other workers have obtained evidence of the presence of 3-indolylacetaldehyde in plants as "neutral auxin" and of its conversion to 3-indolylacetic acid by plant tissues. We attributed our previous failure to isolate 3-indolylacetaldehyde as the product of the amine oxidase-catalysed reaction to the fact that, in the crude preparations of amine oxidase then used, other enzymes were present which catalysed the oxidation of the product of the primary reaction. With the highly purified enzyme preparations now available it should be a relatively simple matter to find out whether the aldehyde is the product of the oxidation of tryptamine, and experiments to test this have been started in view of the possible significance of the reaction *in vivo* and in the hope that the reaction may prove to be a simple method of preparing the aldehyde. In the presence of catalase, purified amine oxidase gives a quick O_2 uptake until 0·5 mol. O_2 has been taken up and 0·5 mol. NH_3 formed for each mol. of tryptamine. Thereafter the O_2 uptake is slow. No CO_2 is formed during the reaction, and colorimetric tests suggest that the indole ring remains intact in the oxidation product. From the reaction mixture a 2 : 4-dinitrophenylhydrazone and a dimedone derivative have been isolated in yields of 20–30 per cent, suggesting that a carbonyl compound, probably an aldehyde, is one of the main products. In both cases

https://doi.org/10.23637/ERADOC-1-76

the melting points are only slightly lower than those recorded for the corresponding derivatives of 3-indolylacetaldehyde. Further oxidation of the reaction products with silver oxide results in the formation of a compound resembling 3-indolylacetic acid in colorimetric tests and in investigations by paper chromatography.

We have shown previously that hydrogen peroxide is formed in the reactions catalysed by plant amine oxidase. This is a characteristic of flavoprotein enzymes, but attempts made in the present work to demonstrate that a flavin is the prosthetic group of the enzyme have so far been unsuccessful. Evidence has, however, been obtained that a metal may form a part of the prosthetic group. Thus the enzyme is inhibited by sodium diethyldithiocarbamate, potassium ethyl xanthate and salicylaldoxime, which are usually considered as specific inhibitors of copper-containing enzymes. In preliminary experiments the three plant amine oxidase preparations so far examined for heavy metals have been found to contain 0·05–0·06 per cent copper and 0·03–0·04 per cent manganese based on the protein content. No proof has yet been obtained that the presence of either of these metals is essential for the activity of the enzyme.

Properties of Fractions from Leaves Infected with Tobacco Mosaic Virus

(N. W. Pirie)

Measurements have been made of the amount of antigen, reacting specifically with anti-TMV serum, in the supernatant fluids and pellets after centrifuging extracts of infected leaves at 30,000–60,000 g. The ratio between the two types of antigen varies with the strain of virus used, the age of the inoculated plant, the duration of infection and the cultural conditions, but we never find more than one-tenth of the whole in the unsedimented fraction.

After various treatments, such as heating to 60° and incubation with proteolytic enzymes, the unsedimented material aggregates and then sediments normally. Neither in the native nor aggregated state does it carry an amount of infectivity larger than could be explained by contamination. Similar material has been made in other laboratories, and there are claims that it is free from P and so from nucleic acid. Some of our preparations have a lower P content (0·2 per cent instead of 0·5 per cent) than that characteristic of the sedimentable fraction, but we have not encountered a serologically active fraction free from P.

TMV preparations made by ultracentrifugation carry several enzyme activities and P in states of combination other than firmly bound ribonucleic acid. The obvious interpretation is that this is a consequence of contamination, but other interpretations are being explored (with F. C. Bawden).

Leaf Ribonuclease and Nucleoprotein

(M. Holden and N. W. Pirie)

Preparations of leaf ribonuclease have now been got sufficiently free from phosphomonoesterase, phosphodiesterase, deoxyribonuclease and some other enzymes for it to be clear that the enzyme is relatively specific. Our best preparations, however, still carry

https://doi.org/10.23637/ERADOC-1-76

activity against metaphosphate and adenosine triphosphate.* These preparations are as active, weight for weight, as the most highly purified preparations from pancreas, but they are still inhomogeneous.

Improvements have been made in the methods used to prepare nucleic acid from yeast, viruses and normal leaf nucleoprotein and, using these less contaminated preparations as substrates, we have been able to systematize our observations on the kinetics of ribonuclease action. The outstanding differences between enzyme from leaf and pancreas are that the former, as stated in earlier reports, carries hydrolysis farther than the latter and that it is more easily inhibited by several inhibitors. Some of these inhibitors, notably Ca, Mg and Zn, are present in the particles of normal leaf nucleoprotein, and this probably explains some of the anomalies in its behaviour *in vitro*.

Large-Scale Production of Leaf Protein

(D. Fairclough and N. W. Pirie)

Effort has still to be directed more to perfecting machinery than to measuring its performance in use. The pulper, which is the first machine to have been built expressly for this job, works well, and on any material that is worth using we can grind up to 1 ton per hour for the expenditure of 25 H.P. To get good performance, steady even feeding is necessary, and we have perfected an automatic feeder which can be loaded intermittently, but, by a system of relays controlled by the current taken by the driving motor of the pulper, maintains a feed that keeps the pulper working optimally.

The adequacy of pulping is assessed in three ways: by measuring the total amount of protein liberated from the fibre; by measuring the rate at which juice is expressed under standard conditions of pressing; and by measuring the yield of protein that can be attained in practice. Laboratory-scale equipment has been built for the first two operations and a full-scale press for the last.

This press is the first of its type, and in it a piston presses the pulp on a perforated conveyor which is only moved forward between the piston strokes. It runs automatically, and the intensity and duration of pressure are adjustable. We are still measuring the performance of this press with different thicknesses of pulped material on the belt so as to find what increase in juice expression is got by diminishing the rate of working. The press has also been used on grass that has not been pulped but simply exposed to steam sufficiently to destroy osmotic control in the leaf cells. From steamed leaves, unlike raw leaves, it is easy to press two-thirds of the water with a variable but small loss of sugars, amides, etc. In dried grass manufacture it may well be that, in spite of this loss, it would be more economical to press out water rather than to dry it off.

Our technique for handling the protein-containing juice coming from the press and for processing the protein is still relatively crude, and the possibilities of improving it are being actively explored. In agreement with earlier work, both in the laboratory and on a

* Pierpoint has now removed these two activities from the preparation.

https://doi.org/10.23637/ERADOC-1-76

large scale (cf. " Large-Scale Production of Edible Protein from Fresh Leaves " in *Report of the Rothamsted Experimental Station for 1952*), we find that from leaves containing more than 18 per cent of protein it is easy to get 30–40 per cent of the protein in one extraction and up to 60 per cent by two extractions.

The Properties of the Resistant Parts of Fungal Mycelium which May Accumulate in Soils

(M. V. Tracey)

It has previously been reported that the mycelium of a number of soil fungi cannot be broken down completely by the enzymes that have so far been investigated. In part this appears to be due to the protection of a varying amount of the chitin that they contain from attack by chitinase. The chitin of the mycelium of the mature puffball is completely resistant to enzymic attack, and it has therefore been used as a suitable material for further study. Efforts were made to isolate micro-organisms, from soil collected from a site at which puffballs multiply, that would decompose the material. Although a large number of samples were examined by the soil-plate method, no effective organisms were found. Recently, decomposition was found to have been begun in a water suspension of the material that had been exposed to the air. Serial transfers with puffball mycelium as sole energy source of the organisms present have been successful, and it is hoped that the organism or organisms responsible will be isolated. An examination of their effect on the material may then throw light on its nature. Chemical studies have also been undertaken in the hope of being able to differentiate chemically between chitin and an insoluble material with a high glucosamine content that is present in amounts of up to 18 per cent (calculated as a glucosamine polymer) in the puffball. The material is not acetylated, probably occurs in a number of other fungi and may be of importance in the protection of chitin from enzymic attack. It is hoped to investigate its relation to " lycoperdin ".

The editing of Volume II of *Modern Methods of Plant Analysis* has been completed, and the remaining volumes are almost entirely in first proof form. A contribution on plant ureides has been prepared for Volume IV. It is believed, as a result of the literature survey entailed, that these compounds may prove of considerable importance in the protein metabolism of legumes and some other groups of plants, and may perhaps be of interest in nucleic acid metabolism.

https://doi.org/10.23637/ERADOC-1-76