ROTHAMSTED RESEARCH

# Rothamsted Report for 1951

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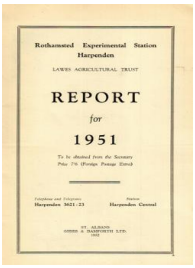


## Biochemistry Department

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# BIOCHEMISTRY DEPARTMENT

By N. W. Pirie

## Nucleoproteins from Normal and Virus infected Leaves

(M. Holden and N. W. Pirie)

Factors influencing the infectivity of a tobacco-necrosis virus are being studied with particular attention to the conditions under which there are losses of activity during a few days' storage at 4°. Preparations have approximately the same initial infectivities whether they are made by ultracentrifuging leaf sap once or twice either directly or after dilution. When compared again after an interval of 1-4 days twice centrifuged preparations retain their infectivity substantially unaltered but the infectivity of those centrifuged only once falls, especially if the sap is diluted before centrifuging. This fall can be prevented by the addition of azide. Thus far the phenomena are invariably reproducible so long as leaves in a more or less standard physiological state are used. Many preliminary treatments of the sap, e.g. short periods of ageing before the first ultracentrifugation, dialysis, freezing, shaking with chloroform have given preparations that after only one ultracentrifugation, retain their infectivity. The precise nature of this phenomenon is still obscure, but work proceeds on the hypothesis that the inactivation is due to an unstable enzyme system associated with the sedimentable normal nucleoprotein of the leaf, acting on residual unsedimentable substrate(s) in preparations that have only been centrifuged out once. The system is of interest because the unrecognized occurrence of this type of inactivation can vitiate studies of the relative infectivities of virus preparations made from plants that have been treated differently and also because, if any similar processes go on in the leaf they must influence the course of a virus infection. (In collaboration with F. C. Bawden.)

The state of combination of phosphorus in the normal tobacco leaf continues to interest us and four fractions have been separated : (1) P soluble in cold dilute acid, (2) lipid P, (3) ribonucleic acid P, (4) deoxyribonucleic acid P. The distribution of P in the four fractions was determined in fibre, chloroplast suspensions and precipitates from sap.

The P soluble in cold dilute acid is inorganic phosphate and organic compounds of low molecular weight. Most of the lipid P is not extractable with ethanol-ether until after the tissue has been extracted with acid. Ribonucleic acid (RNA) can be extracted in the form of nucleotides by soaking leaf tissue in dilute perchloric acid at 16° overnight. Deoxyribonucleic acid is not soluble under these conditions, but is brought into solution when the tissue is incubated in perchloric acid at 37° overnight. The separation of RNA and DNA is not perfect, but the RNA fraction is substantially free from DNA though the DNA fraction contains some RNA.

Colorimetric methods, which have been used for determining DNA in animal tissues, have been investigated for their applicability to plant material, In these methods the interference caused by substances such as pectin and its breakdown products, which

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are likely to be present in most plant extracts, has been studied. All the methods have some disadvantages, but when DNA-containing extracts can be prepared that are free from RNA it should be possible to get good agreement between values for DNA determined colorimetrically and by P estimation.

The different forms of P in the leaves of several other species of plants, and in tobacco leaves of different ages have been compared. The differences between various species are no greater than between leaves of different ages in the same species. Small young leaves have a high P content and during expansion although the total amount of P present increases, when it is expressed on a dry matter basis there is a marked fall. Nucleic acids form a greater proportion of the total P in young leaves than in old, which have a higher lipid P content. The amount of deoxyribonucleic acid P per leaf remains constant during expansion. Of the total P in the tobacco leaf about 30 per cent is in the form of ribonucleic acid P ; two-thirds of this is associated with the insoluble fibre fraction. Deoxyribonucleic acid P accounts for about 7 per cent and lipid P for 15 per cent of the total P in the leaf.

When leaf fibre or a chloroplast suspension is incubated, up to 80 per cent of the total P becomes soluble ; this liberation is activated by salts, especially citrate, and there is every reason to think that it is due to the fission of nucleoprotein which also occurs as a sedimentable component of fresh sap. Using purified nucleoprotein preparations an attempt is being made to discover whether ribonuclease alone can bring about the fission or whether preliminary separation of nucleic acid from protein by another enzyme is necessary. Leaf ribonuclease does not catalyse precisely the same action as pancreatic ribonuclease, for the former converts the whole of a sample of yeast nucleic acid into an acid soluble form, whereas the latter leaves a resistant core. So that it can be used as a tool for further investigation of the nucleoproteins of the leaf, we have begun to fractionate leaf ribonuclease.

### Technological Production of Leaf Protein

(M. L. Barnes, D. S. Miller and N. W. Pirie)

This work has been carried out for three years at the Grassland Research Station on a grant to Rothamsted. It is now becoming part of the work of the Grassland Research Station and will no longer be financed through Rothamsted.

During the year we have made large quantities of protein from various crops for chicken feeding experiments and have supplied leaf residue from which much of the protein has been removed, to the Rowett Research Institute. It will be used there for sheep feeding experiments.

Our arrangements for grinding leaves are fairly satisfactory, but we still have trouble in pressing out the juice from the ground mass. Several improvements have been made during the year and a press working on an entirely new principle is now being made for us at the National Institute for Agricultural Engineering.

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## Manganese Oxidation and Enzyme Systems producing Hydrogen Peroxide

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

Work has been continued on oxidation of $Mn^{++}$ by plant extracts and on enzyme systems producing $H_2O_2$ which may be associated with this oxidation. The oxidation of $Mn^{++}$ has not yet been demonstrated in the plant *in vivo* under normal conditions of manganese supply. In experiments with plants grown in culture solutions of high $Mn^{++}$ content, colorimetric evidence has been obtained suggesting the accumulation of higher valency forms of manganese in the tissues. The work on the reconstruction from partially purified enzyme preparations of the type of system causing oxidation of $Mn^{++}$ in plant extracts has now been completed. When the oxidation is brought about by peroxidase systems with added $H_2O_2$ the oxidation product, in orthophosphate media, is reduced by the $H_2O_2$ as rapidly as it is formed. If, instead of adding $H_2O_2$, enzyme systems such as the amine oxidase D-amino-acid oxidase are used to maintain a suitably low concentration of $H_2O_2$, the oxidation product accumulates quantitatively. Under these conditions it would therefore be available in the plant for the oxidation of metabolites other than $H_2O_2$. During the course of these experiments it was found that peroxidase, coupled with plant α-hydroxy-acid oxidase preparations with lactate or glycollate as substrate, oxidized $Mn^{++}$, suggesting that α-hydroxy-acid oxidase systems produce $H_2O_2$. This has now been demonstrated by more conventional methods. Using α-hydroxy-acid oxidase preparations of low catalatic activity with lactate and glycollate as substrates, $H_2O_2$ production can be shown by the effect of catalase on the $O_2$ uptake. $H_2O_2$ does not accumulate. In absence of catalase it reacts non-enzymically with the products of the enzyme catalysed reactions—pyruvate and glyoxylate—to give acetate and formate respectively.

While evidence is accumulating that $Mn^{++}$ is oxidized in higher plants, it is not yet known what plant metabolites reduce the oxidation product. In this connection preliminary results suggest that the known oxidation of dihydroxymaleate in presence of $Mn^{++}$ and peroxidase or compounds of peroxidatic activity depends on the oxidation of $Mn^{++}$ and the known oxidative decarboxylations of oxaloacetate and oxalosuccinate by plant tissue preparations are due to the presence of peroxidase and likewise depend on oxidation of $Mn^{++}$.

## The Properties of the Resistant Parts of Fungal Mycelia which would accumulate in Soil

(W. R. Smithies and M. V. Tracey)

Samples of *Penicillium griseofulvum* mycelium have been analysed with particular reference to the macromolecular components—proteins, carbohydrates and chitin. The effect of autolysis on the composition of this mycelium is being investigated. An air-dried sample which had suffered subsequent autolysis in saturated thymol solution for more than 12 months had a diminished nitrogen and protein content, and the protein was more easily dispersed in water than that from unautolysed air-dried mycelium. The carbohydrate

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and chitin contents are less affected and this is probably due to the relative instability to drying of the carbohydrases and chitinase compared with the proteases. Lignin-like substances, that is material insoluble in strong $H_2SO_4$, have been shown to occur in many fungi, though none was detected in the *P. griseofulvum* mycelium examined. Several specimens of dark bracket fungi have been collected locally and one of these, *Ganoderma applanatum*, contains about 30 per cent of this material and it is under investigation. Water soluble polysaccharides giving viscous solutions at low concentrations have been isolated from a number of higher fungi and are being studied. The changes in composition occurring during the ripening of the fruit body of *Lycoperdon pyriforme* are being followed as in this fungus a remarkably rapid and extensive series of changes result in the breakdown of much of the structure of the fruit body. During this process a powerful chitinase can readily be prepared from material in the right stage of ripening. The spores and outer walls of the fruit-body resist the action of the hydrolytic enzymes and it is hoped to examine the basis of this resistance to enzymic attack. Using the methods developed in this department for the estimation and detection of chitinase and cellulase we have made a preliminary survey of the enzymic apparatus of a number of soil organisms. Seventeen species of earthworms were examined and all found to possess cellulase. Twelve of these were tested for chitinase and found to have it. It is tentatively concluded that these enzymes are produced, in part at least, by the earthworms themselves, though the importance of the bacterial flora of the intestine must not be forgotten. Work done on the enzymes of nematodes parasitic on plants and on fungi is described in the report of the Nematology Department. It may be added here that neither cellulase nor chitinase were detected in the non-parasitic eel worm of vinegar, *Turbatrix aceti*. It is hoped to continue this work on nematodes in collaboration with the Nematology Department and to survey the occurrence of the two enzymes in common soil bacteria and protozoa in collaboration with the Microbiology Department.

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