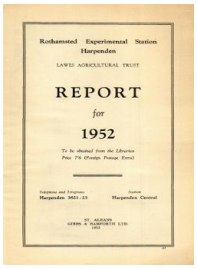
ROTHAMSTED RESEARCH

Report for 1952

Full Table of Content



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MANGANESE OXIDATION IN HIGHER PLANTS *IN VIVO*

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

Last year the oxidation of manganese was the subject of a Special Review in the Report. Work has continued on pea plants (*Pisum sativum* L.) in water culture experiments at high Mn^{++} concentrations and by injection of solutions of manganese sulphate. In both types of experiment a brown colour develops in the stem, more particularly at the nodes. This colour is in part due to the deposition of high oxides of manganese. The affected tissues give an instantaneous blue colour with benzidine and the brown substance can be extracted from the tissues with neutral solutions of pyrophosphate. The extracts so obtained give an ultra violet light absorption curve closely resembling that given by solutions of manganipyrophosphate. Some indication has also been obtained of oxidation of Mn^{++} in the leaves of the plants. In this connection it has been shown that suspensions of washed chloroplast material oxidize Mn^{++} in light, but not, or to a much less extent, in darkness. To demonstrate Mn^{++} oxidation in plants *in vivo* by the technique described toxic concentrations of Mn^{++} are necessary. It has already been shown that the oxidation takes place *in vivo* with physiological concentrations of Mn^{++}. At these concentrations of Mn^{++} *in vivo* it is possible that the Mn^{++} oxidation product is reduced by plant metabolites as fast as it is formed. The results suggest that the symptoms of manganese toxicity may be due, at least in part, to deposition of higher oxides of manganese in the tissues.

THE OXIDATION OF PHENYLACETALDEHYDE AND INDOLEACETIC ACID BY PEROXIDASE AND Mn^{++}

(R. H. Kenten)

The system in pea-seedling sap which catalyses the oxidation of phenylacetaldehyde has been studied. This consists of a thermolabile factor which appears to be a peroxidase and a thermostable factor which can be replaced by Mn^{++}. A system with similar properties to that in pea-seedling sap can be constructed using horseradish peroxidase and Mn^{++}. The oxidation when catalysed by either pea-seedling sap or horseradish peroxidase and Mn^{++} proceeds with the formation of benzaldehyde and formic acid as two of the products, while the formation of H_2O_2 as an obligatory intermediate is suggested by the inhibiting effect of catalase. The mechanism of the reaction is obscure but the results suggest that manganese oxidation is involved in this reaction. It was previously suggested by analogy with the known aldehyde oxidases that indolacetaldehyde might be oxidized by the system to indoleacetic acid (IAA). If indoleacetaldehyde is oxidized in a

https://doi.org/10.23637/ERADOC-1-74

similar way to phenylacetaldehyde the main oxidation would be indolealdehyde and not IAA.

The system in pea-seedling sap which catalyses the oxidation of phenylacetaldehyde bears a marked resemblance to the known IAA oxidase of pea epicotyl and bean roots. This enzyme has been variously reported as an Fe-protein, a Cu-protein, and a flavo-protein-peroxidase system. In this connection it has been shown that horseradish peroxidase preparations alone will catalyse the oxidation of IAA, while peroxidase plus Mn++ is a more efficient catalyst of the reaction. The oxidation of IAA by peroxidase and Mn++, like the known IAA oxidase, leads to the production of CO_2 and a carbonyl compound and is completely inhibited by 0·001 M-diethyldithiocarbamate.

Purification and Properties of the Amine Oxidase of Pea Seedlings *Pisum sativum* L.

(P. J. G. Mann)

The amine oxidase of pea seedling extracts has now been purified several hundredfold. The final preparations obtained are about fifty times as active in catalysing the oxidation of putrescine, as recently described preparations of the diamine oxidase of animal tissues. These enzyme preparations catalyse the oxidation not only of the amines specified in a previous report (1950) but also that of D- and L-lysine. Ornithine also is oxidized, though at a very slow rate. The greater part of the original activity of the extracts towards putrescine is recovered in the final enzyme preparations, but much of the activity towards histamine, the phenylalkylamines, tryptamine and L-lysine is lost. This lost activity can be restored by adding peroxidase, equivalent in amount to that present in the original extract, to the amine oxidase preparation. This effect of peroxidase is, with some of the substrates at least, due to its catalysis of the oxidation of the primary reaction products. The addition of trace amounts of Mn++ produces a similar effect. It is not yet clear whether this effect of Mn++ is dependent on the residual peroxidase activity of the amine oxidase preparations. In the course of the fractionation of the extracts the bulk of the peroxidase is separated from the amine oxidase and preliminary experiments suggest that the method used for this separation may form the basis of an improved method of preparation of peroxidase.

The results so far obtained suggest that L-lysine is oxidized by the amine oxidase at the E-amino group and that oxidation leads to intra-molecular condensation to a ring compound. Evidence for cyclization has also been obtained with cadaverine, putrescine and L-ornithine. From cadaverine a derivative of Δ^1piperidine has been isolated in good yield and colorimetric tests suggest the presence of Δ^1 pyrroline compounds in putrescine and ornithine reaction mixtures. In view of the results with cadaverine it appears probable that the ring compound formed from L-lysine is Δ^1 piperideine-2-carboxylic acid. In this connection it is of interest that the corresponding saturated compound, piperidine-2-carboxylic acid, has recently been isolated from higher plants.

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Metabolism of Tryptophan in Plants

(G. H. Wiltshire)

The essential aminoacid tryptophan is known to be a source of the vitamin nicotinic acid in animal tissues. There is some evidence that a similar conversion occurs in some plants, but the intermediate steps have not been demonstrated.

A fairly rapid oxidation of tryptophan was found in pea seedling extracts. The tryptophan is oxidized not by oxygen but by hydrogen peroxide generated in a "coupled reaction," e.g. the oxidation of aliphatic amines by amine oxidase.

1. $R{\cdot}CH_2{\cdot}NH_2 + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{amine}} R{\cdot}CHO + NH_3 + H_2O_2$

2. $C_{11}H_{12}O_2N_2 + H_2O_2 \xrightarrow[\text{peroxidase}]{\text{(tryptophan)}} H_2O + \text{coloured products}$

The enzyme catalysing reaction 2 which uses hydrogen peroxide to oxidize tryptophan was identified as peroxidase. This enzyme is already known to oxidize many aromatic compounds. Experiments are in progress to establish the nature of the products of reaction 2.

Leaf Ribonuclease

(M. Holden and N. W. Pirie)

Ribonuclease is present in the leaves of all species that have been tested and older leaves have higher activity than young. Purified preparations have been made from tobacco leaves and pea seedlings by precipitation with ammonium sulphate and acetone. The most active preparations from pea seedlings have been purified about one-hundredfold on a protein-nitrogen basis. The enzyme differs from pancreatic ribonuclease in its properties and its action on ribonucleic acid. The pH optimum on yeast ribonucleic acid is at about 5·6 compared with 7·7 for pancreatic ribonuclease. Although more thermostable than many enzymes the leaf ribonuclease is not so stable as the pancreatic enzyme. The action of pancreatic and leaf ribonucleases on ribonucleic acid (RNA) preparations made from yeast, uninfected tobacco leaves and tobacco mosaic virus has been compared. All the preparations are hydrolyzed at about the same rate, and when the action of pancreatic ribonuclease has gone to completion a "core" is left and only about 30 per cent of the phosphorus becomes unprecipitable with uranyl nitrate in tricholaracetic acid whereas leaf nuclease destroys completely the precipitability by this reagent. Leaf nuclease is able to break down the core left by the action of pancreatic ribonuclease on yeast RNA.

Most of these experiments were made on solutions containing about 4 g. of nucleic acid per litre; this is a lower concentration than that generally employed in studies of ribonuclease but even so it is unphysiologically high. Some progress has been made in the study of more dilute solutions so that the results can be related to those in which the substrate is the microsomal nucleoprotein from uninfected leaves. The position is still by no means clear but some of the differences that had been noticed between the kinetics of hydrolysis of leaf nucleoprotein and yeast nucleic acid are clearly

https://doi.org/10.23637/ERADOC-1-74

due to the different concentrations at which the two processes had hitherto been studied.

The Infectivity of Tobacco Necrosis Virus
(N. W. Pirie)

Work on the nucleoprotein of the normal leaf and the changes that it undergoes sheds considerable light on the factors that affect the initial infectivity of preparations of the Rothamsted strain of Tobacco necrosis virus and the persistence of infectivity under different conditions of storage. This problem has been tantalizing us for three years with striking but generally unreproducible results. We have now defined experimental conditions that introduce uniformity. The treatments that promote destruction of the normal nucleoprotein, ageing in the sap, treatment with chloroform, dialysis, etc. result in a relatively stable preparation whereas those that stabilize the normal protein, dilution of the sap, very rapid cold working, additions of azide, etc. give a preparation that can be unstable. Whether or not it is unstable depends on the other components of the fluid in which it is stored. The one to which we have recently devoted most attention is ascorbic acid. When the normal concentration in sap is increased about fivefold by the addition of 1 g. per litre, the resulting virus preparation is both relatively uninfective and unstable, but when added to most types of preparation, ascorbic acid acts as a stabilizing agent. Preparations made in the presence of azide are exceptions for they lose infectivity more rapidly in the presence of ascorbic acid than in water. The hypothesis with which we relate these phenomena is that the products of an enzyme action, perhaps hydrogen peroxide, is the inactivating agent and the juxtaposition of enzyme and virus together with the presence in the system of a substrate for the enzyme or a substance that can protect the virus from the products of the action, control the final infectivity. An elucidation of this mechanism should go far to explain the origin of the non-infective anomalous nucleoprotein in infected leaves to which we constantly call attention. (In collaboration with F. C. Bawden.)

Technological Production of Leaf Protein
(N. W. Pirie)

Little progress has been made during the past year in this work, which is done in collaboration with the Grassland Research Station, and application has been made to transfer that part of the work in which we are particularly interested, to Rothamsted.

The Properties of the Resistant Parts of Fungal Mycelia which would accumulate in Soils
(W. R. Smithies and M. V. Tracey)

The action of enzymes on a sample of mycelium of *Penicillium griseofulvum* has been studied. Autolysis of an air-dried sample causes a loss of about 60 per cent of the protein, 20 per cent of the chitin and 30 per cent of the carbohydrate. Subsequent treatment with "Trypsin" (extract of pancrease) leaves only about 15 per cent of the original protein insoluble. Snail digestive juice brings over 90 per cent of the residual chitin into solution as

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N-acetylglucosamine, and about 80 per cent of the carbohydrate. Pancreatic amylase, and the several fungal enzyme preparations tried, had in contrast, practically no action on the mould carbohydrate. It seems therefore that, for this particular mycelium, significant amounts of protein and carbohydrate are not available to enzymic attack or are resistant.

The composition of two wood rot fungi, *Polystictus versicolor* and *Polyporus sulphureus* has been determined. Neither, however, contains any lignin-like substances, in contrast to the specimen of *Ganoderma applanatum* mentioned in last year's report.

A method has been developed for the estimation of glucosamine in the presence of amino acids and sugars which interfere, in general causing high values, with the conventional procedure of Elson and Morgan. It has been found useful in the examination of mycelium and plant extracts.

Evidence has been secured that the amino sugar formed on the hydrolysis of water-insoluble portions of fungal mycelium is not all derived from chitin. Removal of the non-chitinous glucosamine containing polymer increases the ease with which the remaining chitin is attacked by chitinase. It appears that glucosamine derived from chitin may account for from 20-80 per cent of the glucosamine present. In *Lycoperdon pyriforme* the " chitin glucosamine " appears to be about 20 per cent of the total, in *Botrytis fabae* about 35 per cent, in *Daldinia concentrica* about 60 per cent, and in *Penicillium griseofulvum* 80 per cent or more. A search for enzymes capable of breaking down the material giving rise to " non-chitin glucosamine " is being begun. A large number of bacteria have been examined for the presence of chitinase and the production of this enzyme has been found to be widespread. This property appears to be of interest in the identification of bacteria by biochemical tests and the work is being pursued with the assistance of members of the staff of the National Collection of Type Cultures. Three species of amoebae found in soil have been examined so far, with the help of the Soil Microbiology Department, and all were shown to produce chitinase. The observation by Whitaker that cellulase activity is increased in the presence of protein has been shown to be true for chitinase and since the addition of small amounts of protein may quadruple the observed chitinase activity it is likely to prove of great value in studying chitinase production by soil organisms. Two water-soluble polysaccharides isolated from *Phallus impudicus* and *Mutinus caninus* have been shown to be polyuronides. They are not identical with pectic acid as there are marked differences in the ease with which they and pectic acid are attacked by a number of fungal and animal enzymes.

Decomposition of Naphthalene and Chloronaphthalene by Soil Bacteria

(G. H. Wiltshire)

Collaborative work on the breakdown by bacteria of naphthalene and chloronaphthalene has been carried out with N. Walker and three strains of bacteria have been isolated. This work is described in the report of the Soil Microbiology Department.

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