ROTHAMSTED RESEARCH

Report for 1949

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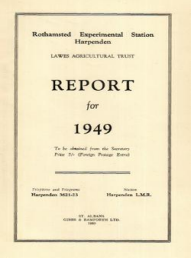

Biochemistry Department

N. W. Pirie

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BIOCHEMISTRY DEPARTMENT

By N. W. Pirie

Investigation of Leaf Components

Several years of work on the effect of enzymes from various sources has now been rounded off sufficiently for it to be written up. The essential features were summarised in last year's report.

By following the reduction in viscosity of a solution of a soluble cellulose derivative under standard conditions, Tracey has detected the presence of cellulase in saps from a number of plants. The enzyme is present in very small amounts in tobacco leaf sap, its concentration often being less than a one hundred thousandth that in snail digestive juice (the usual source of cellulase used in this department). In tobacco root sap the concentration may be five or ten times as great. It appears that the enzyme is relatively more abundant in young leaves than in old. By using large amounts of fresh active root sap it has been possible to show that finely dispersed cellulase is also attacked by the enzyme.

The readily sedimentable phosphorus-containing protein that is a characteristic component of the fresh sap of young tobacco leaves has now been found to contain several enzymes. These will not only act on extraneous substrates e.g. phosphate esters, but also bring about the denaturation of the protein itself and the liberation from it of nucleotide and, subsequently, the dephosphorylation of the nucleotide.

Large scale preparation of leaf protein has been started at the Grassland Improvement Station where a mill capable of handling up to 2 tons of fresh leafy material has been installed. This works satisfactorily, and we have now sufficient skill in its use to be able to grind any of the leaves grown in normal agricultural practice and to liberate about half the protein.

Investigations on tobacco necrosis virus have continued along the lines set out in last year's report and some progress has been made towards getting conditions defined, so that the results will be repeatable. Some of the important variables have been recognised, but the frequency with which unexpected activations, or inactivations, occur suggests that we have not recognised all of them.

Oxidation of Mn by Peroxidase Systems in Plant Extracts

The system consists of peroxidase, and a peroxidase substrate. Using partially purified peroxidase preparations, the effect of variation in the peroxidase, phenolic substrate and H_2O_2 concentration has been studied. The oxidation of Mn can be demonstrated at Mn concentrations likely to be present *in vivo*, and with a few μg. of peroxidase. The Mn probably reduces the oxidised peroxidase substrate, and thereby is itself oxidised. This involves the substrate in a cycle of oxidation and reduction. The catalytic activity of the substrate is demonstrated by the fact that under suitable conditions 1 μg. of p-cresol produced 605 μg. Mn_2O_3.

Preliminary evidence has been obtained that the oxidized Mn is reduced by plant metabolites. This is an agreement with the

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hypothesis previously put forward that a Mn oxidation reduction cycle is responsible for the effect of Mn on plant respiration.

The oxidation of Mn *in vivo* by this system depends on the production of H_2O_2 by the plant tissues. It is known that H_2O_2 is formed in several oxidation reactions catalyzed by enzymes, e.g. xanthine oxidase and amino acid oxidase. Preliminary evidence has been obtained that H_2O_2 formed by these enzymes may be used by peroxidase systems to bring about the oxidation of Mn.

The possibility that the peroxidase system can oxidize metallic ions or complex ions other than those of Mn is being investigated.

Under certain conditions the addition of small amounts of salts of Mo, V and W have been found to accelerate the rate of oxidation of Mn by peroxidase systems. There is evidence that this is due to the enzyme catalyzed formation of peracids, e.g. the formation of permolybdate from molybdate. These experiments may throw light on the mechanism of peroxidase action and the physiological role of Mo.

The rate of oxidation of ferrocyanide by H_2O_2 is increased by peroxidase alone, but peroxidase together with suitable substrate produces a much more striking increase in the rate of oxidation. The oxidation of ferrocyanide is accompanied by consumption of H^+, and may be followed manometrically by CO_2 uptake in bicarbonate medium in an atmosphere of $N_2 + CO_2$.

Hydroquinone—and Hydrosulphite—Soluble Manganese of Organic Soils

It has been suggested that manganese deficiency occurring in soils with total manganese contents adequate for plant growth is due to the presence of the soil manganese higher oxides in unreactive forms. The highly reactive and potentially readily available fraction has been defined as the fraction dissolved by 0·2 per cent. hydroquinone in M-ammonium acetate at pH 7, and a moderately reactive fraction as that dissolved by hydrosulphite under the same conditions. Heintze and Mann have shown that manganese extracted from neutral or alkaline organic soils by M-ammonium acetate containing 0·2 per cent. hydroquinone at pH 7 may represent only a small percentage of the soil manganese higher oxides reduced by hydroquinone during the extraction. This is due to the retention by the soils of part of the divalent manganese formed by reduction. The amount of manganese retained in this way depends in general on the organic content of the soil. Results with the manganese minerals, pyrolusite, hausmannite, and manganite support the view that under certain conditions hydroquinone and hydrosulphite may be suitable reagents for differentiating between manganese higher oxides of different reactivity. But since hydrosulphite, unlike hydroquinone, partially prevents retention of divalent manganese by soils, differences between hydrosulphite and hydroquinone soluble manganese, on organic soils in particular, may be due not to the reduction of manganese higher oxides of different reactivity but to differences in the amounts of divalent manganese retained by the soils.

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Resistant Components of Soil Fungi

Work started in October on the chemical nature of the mycelia of some representative and easily available fungi. This will later be extended to the examination of those that are prominent in soil to see whether it is likely that they contribute significantly to the building up of "soil organic matter."

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