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Report for 1948

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Rothamsted Experimental Station
Harpenden

LAWES AGRICULTURAL TRUST

REPORT
for
1948

Biochemistry Department

N. W. Pirie

N. W. Pirie (1949) *Biochemistry Department ;* Report For 1948, pp 57 - 60 **- DOI: https://doi.org/10.23637/ERADOC-1-70**

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BIOCHEMISTRY DEPARTMENT

By N. W. Pirie

Our work on the enzymic decomposition of leaf fibre has been co-ordinated during the year and we hope soon to be able to publish a comprehensive report on the carbohydrases that are involved, and on the factors that influence their action.

Analytical Methods

The development of methods of analysis suitable for application to enzymic digests of leaf fibre has been continued by Tracey. The rapid method for the determination of uronic acids reported on last year has proved satisfactory. A colorimetric method for the estimation of pentoses in the presence of large amounts of hexose and uronic acids has been developed and is now in use. It has been found that the pentose content of tobacco fibre is low—about 2 per cent. of the dry weight. He hopes to develop a method for the estimation of galactose in the presence of large quantities of glacturonic acid. If this is successful it should be possible to obtain figures for all the sugars present in enzymic digests of leaf fibre, and. as a consequence, perhaps, understand better the action of the snail enzymes in liberating virus from fibre.

The enzymes present in the digestive juices of the snail have been further studied both with regard to the range of substrates they are able to attack and to their activity on individual substrates. Tracey has developed a very sensitive method for the estimation of small quantities of cellulase, based on measuring the reduction in viscosity of a soluble cellulose derivative by the enzyme. Even with this method, which can detect a 1 in 100,000 dilution of snail digestive juice, no cellulase activity has been demonstrated in leaf extracts or leaf fibre.

Kinetics of Carbohydrases

M. Holden has continued to work on the effect of salivary amylase, trypsin, purified polygalacturonase, the digestive juice of the snail, *Helix aspersa*, and a number of fungal extracts on leaf fibre. The extract from *Aspergillus aureus* and two commercial enzyme preparations Pectinol 10M and Enzyme 19AP (Rohm and Haas) are as effective as snail digestive juice in breaking up the fibre and releasing soluble carbohydrate material. Factors, such as pH, concentration of salts, and concentrations of enzyme and substrate, affecting the rate and extent of the action of the various enzymes, have been studied. Fine grinding of the fibre in the triple roller mill, before incubation with enzymes, was found to increase the rate of action of the enzymes but made little difference to the total amount of carbohydrate liberated. Removal of calcium from fibre greatly increased the rate of action of snail digestive juice and fungal enzymes. With purified polygalacturonase on decalcified fibre an increase in the total amount of carbohydrate liberated was found. Up to 80 per cent. of the fibre calcium can be removed by treatment at pH5 with an acetate-ammonium chloride solution. Extraction at pH 3 does not increase the amount removed but almost all the calcium remaining after the salt treatment can be brought out by extracting with 0·05N HCl.

https://doi.org/10.23637/ERADOC-1-70

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Oxidation of Manganese by Plant Extracts in the Presence of H_2O_2

Manganese apparently plays a part in plant respiration. Lundegardh found that the O_2 uptake of Mn deficient wheat roots was raised by 155–470 per cent. by the addition of 5×10^{-5} M. $MnCl_2$. Such an effect might be brought about by a system in which the Mn undergoes alternate oxidation and reduction. Satisfactory evidence has been put forward that Mn is oxidized in the higher plants; it can also be oxidized by soil micro-organisms (Mann, P. J. G. and Quastel, J. H.—1946, Nature, **158**, 154).

In preliminary investigations of the distribution of Mn in the plant, Kenton and Mann have obtained evidence that Mn is oxidized by certain plant extracts in the presence of H_2O_2. During the year an investigation of the system has been made with the following results:—

(1) A system which brings about the oxidation of Mn in the presence of H_2O_2 has been demonstrated in horseradish root extracts. Evidence has been obtained that this system exists in other root extracts.

(2) Suitable conditions (i.e. in pyrophosphate or citrate at pH 7) colorimetric evidence has been obtained that the oxidized Mn can be accumulated as a coloured manganic complex. In the case of the horseradish root extract, MnO_2 was isolated by the dismutation of the manganipyrophosphate at weakly alkaline reaction. The oxidation product decomposes N_2H_4 and manometric estimation of the oxidation product have been made by means of this reaction.

(3) Further evidence that Mn oxidation takes place has been obtained by demonstrating an increase in the catalase activity of horseradish extract by the addition of $MnSO_4$. A definite increase in the catalase activity could be demonstrated by the addition of 2·2 μg. Mn.

(4) Studies of the effect of heat, pH, inhibitors, and H_2O_2 concentration have been made. The results suggest that an enzyme is involved. The system is insensitive to low concentrations of cyanide, otherwise the results support the view that the enzyme is a peroxidase. In addition another factor may be necessary.

(5) The hypothesis is put forward that a Mn oxidation-reduction cycle is responsible for the effect of Mn on plant respiration.

The effect of Cu on Mn oxidation in the soil

Lees obtained evidence that Cu is necessary for nitrification in soils. In soils treated with Cu-enzyme poisons e.g. diethyldithiocarbamate, nitrification was inhibited and could be restored by the addition of small amounts of $CuSO_4$. Using the same technique a similar effect of Cu has been shown on Mn oxidation by soils. The partial reactivation of diethyldithiocarbamate inhibited soils has been demonstrated with very low $CuSO_4$ concentrations.

The easily reducible Mn of organic soils

Heintze and Mann have continued their work on the problem of Mn deficiency and have put forward the hypothesis that such

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deficiency occurring on neutral and alkaline soils of high organic matter content and of adequate total Mn content is due to the formation of complexes of divalent Mn with the organic matter which are dissociated to such a slight extent that the Mn in the soil solution is insufficient for the needs of the plant.

The easily reducible Mn (extracted by N. NH_4 acetate containing 0·2 per cent. hydroquinone) forms a much smaller part of the total soil Mn than is the case with mineral soils of low organic matter content. Preliminary results indicate that this is due not to the presence of the Mn in forms resistant to reduction, but, at least in part, to retention by the soil organic matter of the divalent Mn produced on reduction.

The Study of Tobacco Necrosis Virus

This virus has been under more or less continuous investigation by Bawden and Pirie since 1941, but it still proves perplexing. Using a standard method of purification, two or three cycles of ultracentrifugation, and low speed centrifugal clarification, preparations have been made from leaves subjected to a wide range of pre-treatments and the infectivity and chemical, physical, and serological properties of the products have been compared. The age of the infected leaf and the duration of infection do not have a great influence. The conditions under which the leaf, after removal from the plant, and the sap are kept do, on the other hand, exert a large influence. In the leaf most treatments e.g. freezing, wilting and exposure to chloroform vapour, tend to inactivate the virus. In the sap ageing for a few days or freezing or exposure to chloroform cause an activation. This effect is partly due to the removal of unstable normal proteins but our results cannot be explained purely on the basis of dilution of the final preparations to different extents by normal proteins. All infective preparations contain at least three distinct substances, the infective virus, a serologically active sedimentable nucleoprotein that is either a derivative of the virus or a product of the deranged metabolism of the infected leaf, the normal sedimentable nucleoprotein which can also be made from uninfected leaves. An attempt is being made to define the relationship of these substances to one another.

Proteins of normal leaves

All virus preparations are to some extent contaminated with normal leaf protein and these proteins have been investigated from time to time during the last decade. The most interesting is a nucleoprotein with a sedimentation constant in the same range as the viruses which occurs to the extent of 1–2 g per 1 in sap from young leaves. The preparation of those viruses that give small yields is only possible because older leaves, and especially leaves that have matured as a result of virus infection, contain much less of this protein and because it is less stable than the viruses with which we work and dissociates into an insoluble part and an unsedimentable part during rigorous purification. Its properties are being studied in the hope that a convenient method of recognising it as a contaminant of virus preparations may be discovered.

During this year a start has been made on the preparation of leaf protein on a technical scale. Several mills suitable for grinding fresh leaves at about a ton an hour have been tested and the most

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suitable will be installed and operated at the Grassland Improvement Station during 1948. In general the performance of a full sized mill has to be tested, it cannot be deduced from laboratory tests. Stamping mills are an exception and Tracey has measured the amount of work needed to liberate sap from grass and other leaves by impact. Liberation is satisfactory with the expenditure of about 10 horse-power for a ton an hour grinding rate.