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Rothamsted Report for 1947

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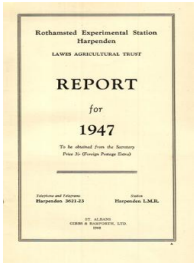

Biochemistry Department

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DEPARTMENT OF BIOCHEMISTRY

By N. W. Pirie

The main work of the Department continues to be an investigation of the composition of the normal and virus infected leaf; we are not primarily concerned with the elementary composition, though this has been investigated during the year, but with the disposition of the various enzymic and structural components of the leaf and their relationship to each other and to the virus that may be present. In work of this type it is always necessary to consider the extent to which the conclusions may be invalidated by changes taking place during the mincing and extraction of the leaves. We have already described the artefacts that can be produced by fine milling, and the demethylation of the leaf pectin by its own pectase. Many leaves, especially those of the Cucurbitaceae, give alkaline extracts when the fibre residue left after expressing the sap is extracted with water. This phenomenon has been studied by M. Holden and she finds that it is due to an apparently non-enzymic reaction between pectic acid, calcium carbonate and phosphate. An understanding of the mechanism will enable us to avoid the inactivation of viruses or enzymes which would result if uncontrolled pH drifts were taking place. Although most plant viruses are not destroyed by proteases the complexes that they form with various leaf components are destroyed. M. V. Tracey has estimated the protease content of a variety of leaves and leaf extracts and finds that the activity present in the leaves normally used in virus work is small. There is no reason to think that this trace of protease has any serious effect on any of the conclusions that we have so far drawn.

The effects of fertiliser treatment and illumination on the susceptibility of plants to infection and on the multiplication of viruses in the infected leaf have been established, mainly by work in the Plant Pathology Department. Holden and Tracey have measured the effects of fertiliser treatments on the composition of tobacco plants grown under the conditions used in virus work. Supplements of nitrogen and phosphorus increased the dry matter, N and P contents of the plants. Nitrogen alone decreased the protease and increased the pectase content whereas phosphorus alone had the opposite effect. The effect of fertilisers on the composition and virus content of tobacco leaves systemically infected with tobacco mosaic virus was studied in a similar way. Under conditions of high phosphorus but without increased nitrogen, virus accounted for four-fifths of the nitrogen associated with the fibre.

In earlier work on the liberation of viruses from leaves some observations were made on the liberation of protein from the leaf residue and the conditions under which fairly complete mechanical liberation was possible were laid down. This work has now been extended by Crook and Holden to some thirty plant species. Wide variations were found in the extractability of the nitrogen in different species. These figures, which will be extended, are both of theoretical interest and of practical value for an investigation of the technical separation of leaf protein which is about to be made.

During the past few years our knowledge of the manner in which viruses are bound in the infected cell has been increased by

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work on plant viruses in Rothamsted and on animal viruses in some other laboratories. Material with properties similar to those of highly purified virus preparations probably exists in the cell but it is accompanied by variable amounts of material that can be converted into purified virus but that is originally in a chemically more complex state. This phenomenon is still being studied with tobacco mosaic virus, tomato bushy stunt, and the Rothamsted strain of tobacco necrosis virus but no unequivocal evidence whether the complex is the primary or secondary form has been found Variable, but often large, amounts of virus are attached to the leaf fibre as well. This is not set free by grinding unless grinding of such an intensity that there is a probability of it bringing about chemical changes is used. The enzymes present in the crop of the snail readily bring about the liberation. An attempt is now being made to find which particular enzyme in this mixture is responsible.

The leaf contains a wide range of structural materials, protein, pectin, cellulose, and the more vaguely defined materials lignin and hemicellulose. The first two are probably not concerned in the anchoring of the bound virus. Lignin also is probably not involved because virus is liberated by enzyme mixtures that are not known to affect it. The roles of cellulose and the hemicelluloses could be disentangled if specific enzymes were available but in the absence of these, evidence can be got from simultaneous estimations of pentose, glucose, galactose and uronic acid. The estimation of these substances in the presence of one another is a matter of some difficulty. Tracey has developed a quick and convenient micro-method from the conventional uronic acid method and has made a more thorough study than had been made before of its specificity. He has also shown that it can be used for the determination of soil uronic acid.

The snail enzyme, although powerful, has defects. It is only readily available in summer and it has not proved possible to purify it; large amounts of nitrogen and carbohydrate have to be added, in the form of snail slimes, to any digest in which its action is being studied. We are investigating various fungus enzymes and get encouraging results with Botrytis and Aspergillus. Some fungus extracts contain predominantly polygalacturonase and our work has been greatly facilitated by a gift of highly purified polygalacturonase from Dr. Lineweaver in California.

Many factors influence the rate of action of these enzymes and we are studying the effects of different pretreatments of the leaf. In this we are, of course, limited by the fact that the ultimate object is knowledge of the manner in which virus is attached to the leaf so that no treatment is useful if it would destroy the virus. Removal of calcium ions from the leaf is a valuable step and so is the simultaneous dehydration and de-fatting that extraction with alcohol-ether mixtures brings about. This has the additional advantage that it gives the leaf a brittle texture that makes grinding very easy.

Although an investigation of the nature of soil organic matter and of the composition of some of the bacteria and funguses that play a part in building up this organic matter is part of the programme of the department, work on this aspect of the biochemistry of soil has not yet begun. Mann, in collaboration with the

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Chemistry Department, has investigated the metal-organic-matter complexes of the soil. This work was prompted by the observation that the manganese in pyrophosphate extracts of organic soils was in the divalent form whereas earlier work with similar extracts from mineral soils had shown that the manganese was in the trivalent form. It proved impossible to decide what proportion of the divalent manganese arose from organic complexes because the pyrophosphate extracts reduced manganese in higher states of oxidation. However, neutral and alkaline organic soils retain added divalent manganese in a form not recoverable by repeated extraction with M ammonium acetate. In some cases the recovery was increased by the addition of low concentrations of Cu, Cd, Ni, or Zn salts to the ammonium acetate. These mixtures also give higher figures for the exchangeable manganese in untreated soil than extraction with ammonium acetate alone. The effect is particularly marked with copper, which appears to catalyse the reduction of the more highly oxidised manganese of the soil. The retention of added manganese is correlated in a general way with the organic matter content of the soil and all these results support the view that the manganese is associated with the soil organic matter.

In attempting to extract metal-organic complexes, three main fractions have been obtained: the first is water soluble after extraction of the soil with sodium chloride, the second is soluble in pyrophosphate, and the third in 2 per cent. sodium hydroxide. Manganese or copper, added as sulphate, during the sodium chloride extraction, was recovered in the subsequent water or pyrophosphate extracts. In the water extracts the metals were still combined with the organic matter.

The results appear to be of agricultural significance since manganese deficiency occurs typically on alkaline organic soils and copper deficiency has frequently been attributed to fixation of copper by the soil organic matter.

Lees has got more evidence for the view already put forward that copper is essential for nitrification. In soils treated with poisons for copper (such as diethyldithiocarbamate) nitrification is inhibited but activity can be largely restored by small amounts of copper sulphate. Higher concentrations of copper and low concentrations of zinc inhibit strongly; these inhibitions depend on the pH and organic matter content of the soil. Lees suggests that at neutral or alkaline pHs metal-organic complexes are formed in which the metal is not readily available to the nitrifying bacteria.

In collaboration with the Microbiology Department Lees has investigated the trace element requirements of cultures of nitrifying bacteria from Rothamsted soil. An outstanding effect is the stimulating effect of iron. Mixtures of copper and iron have no more effect than iron alone, but either removes the inhibitory effect of an unbalanced mixture of other trace elements. A proper balance of elements is more important than the absolute level of any one ion. The effect of many organic substances on nitrification in soil has been tested but none of them have had any pronounced effect under the conditions employed.

During the early part of 1947 N. W. Pirie was absent in U.S.A. and Canada, and he attended the International Cytological Congress in Stockholm in July.