ROTHAMSTED RESEARCH

Report for 1950

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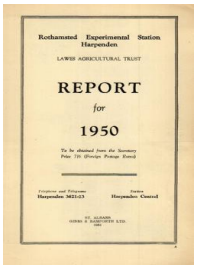

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BIOCHEMISTRY DEPARTMENT

By N. W. Pirie

Nucleoproteins from Normal and Virus Infected Leaves

(E. M. Holden, G. Parker, N. W. Pirie)

We have for long been concerned with the purification of plant viruses, that is with ascertaining what is the minimum chemical complexity needed for a preparation to initiate infection in a susceptible plant. Recently we have become more interested in examining the state in which viruses normally exist in the leaf and in the changes that they undergo during the process of purification. This work has made it more urgent than hitherto to study the unstable components of extracts of the normal leaf, and the controls for much of the experimentation with which we have been concerned during the past 16 years are now, perhaps a little belatedly, being done.

The sap from uninfected young tobacco leaves growing under good nutritional conditions contains up to 3 g./l. of an unstable nucleoprotein that can be sedimented readily on the ultracentrifuge at 50,000 g. Making the usual assumptions therefore, it has a particle weight similar to that of tobacco mosaic virus, and more or less spherical particles of the expected size appear on the electron micrograms. This material disappears from, or at any rate is less readily isolable from, old leaves, ill-nourished leaves, or infected leaves. It is destroyed by a few hours' exposure to leaf sap at room temperature, or by a few days at 0°, but is much more stable in the absence of sap components and of salts.

The protein has an intrinsic biochemical interest because it is a substantial component of the leaf, because it carries with it the enzymes necessary for its own destruction, and because it is responsive to the physiological state of the leaf. By using the known properties of the isolated protein as a guide an attempt is being made to see how much of the phosphorus of the fibrous leaf residues and of the "chloroplast" fraction centrifuged from sap is made up of this material. Similar materials have been prepared from leaves of other plants and when suitable opportunities occur, we hope to examine its composition and behaviour carefully. Because of its instability it will not be a significant constituent of purified preparations of the more stable viruses but, when our present techniques are used, it will contaminate all preparations made by methods designed to separate a virus in its original state.

Some order has now been introduced into our studies of the Rothamsted strain of tobacco-necrosis virus. Extracts from infected leaves contain, besides the normal nucleoprotein mentioned above, a specific non-infective nucleoprotein and material which we look on as virus, partly in an active and partly in an inhibited state. On standing in sap there is at first an increase in the infectivity of the subsequently isolated virus and then a diminution in infectivity. We have examined both phenomena and suggest that much of the virus in the infected but undamaged cell may be non-infective. If this can be substantiated we have in this plant virus a phenomenon comparable to the "masking" that is found with some animal

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viruses and with the lysogenic strains of bacteriophage infected bacteria. The essential difference is that the leaf sap itself contains systems able to bring about the unmasking.

Enzyme Mechanisms involved in Manganese Oxidation

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

It has previously been shown that Mn^{++} can be oxidized by peroxidase systems. The oxidation of Mn^{++} in plants *in vivo* by such systems depends on the production of hydrogen peroxide by the plant tissues. During the year an attempt has been made to demonstrate the presence of H_2O_2 producing enzyme systems in plant extracts and to bring about manganese oxidation by linking these and other known hydrogen peroxide producing enzyme systems with peroxidase systems in presence of Mn^{++}.

Two of the hydrogen peroxide producing enzymes have so far been identified. One is an amine oxidase, the other is an aldehyde oxidase which has not previously been described.

The crude extracts of pea seedlings and partially purified enzyme preparations obtained from such extracts rapidly attack diamines such as putrescine and cadaverine and monoamines such as β-phenylethylamine and indolethylamine. So far it has not been possible to demostrate the presence of this enzyme in extracts of other plants. A quantitative study has been made of the total O_2 uptake during the oxidation of the amines and of the products of oxidation. In these experiments phenyl-ethylamine and putrescine have generally been used as representatives of the mono- and di-amines attacked. The results suggest that the primary attack on the amines is an oxidative deamination according to the equation :

$$R.CH_2NH_2+H_2O+O_2 \longrightarrow R.CHO+H_2O_2+NH_3$$

The formation of hydrogen peroxide during the oxidation of the amines was shown by the effect of catalase on the total oxygen uptake and by the coupled oxidation both of the phenols hydroquinone and p-cresol by peroxidase, and of ethanol in presence of added catalase. The formation of aldehydes during the oxidation was shown by bisulphite titration and, in the case of β-phenylethylamine, by the isolation of phenylacetaldehyde as the 2 : 4-dinitrophenylhydrazone.

An enzyme which catalyses the oxidation of phenylacetaldehyde but not that of benzaldehyde, phenyl propionaldehyde or the n- and branched chain aliphatic aldehydes has been found in extracts of many higher plants. Hydrogen peroxide is formed during the course of the oxidation, but the nature of the other oxidation products has not yet been established. By analogy with the known aldehyde oxidases it appears probable that phenylacetaldehyde would be oxidized to phenylacetic acid. It is possible that the natural substrate of this enzyme is indoleacetaldehyde which is presumably formed by the action of amine oxidase on indolethylamine or from indolepyruvic acid by decarboxylation. Preliminary tests suggest that indoleacetic acid is formed by the combined action of the amine and aldehyde oxidase on indolethylamine, but owing to the

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presence of indoleacetic acid oxidase in the enzyme preparations it has so far not been possible to demonstrate this with certainty.

Some progress has been made in reconstructing from partially purified enzyme preparations the type of system causing manganese oxidation in plant extracts. Manganese oxidation has been demonstrated in pyrophosphate buffers when H_2O_2 producing enzyme systems such as amine oxidase, aldehyde oxidase, xanthine oxidase, and amino acid oxidase are linked with peroxidase systems in presence of Mn^{++}.

The Composition of Fungal Mycelia

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

During the past year work has been started which is designed to throw some light on the nature of soil organic matter. We are approaching the problem by way of a study of chemical nature of the resistant residues of soil inhabiting bacteria and funguses. It seems likely that if we know more about the material from which soil organic matter is derived the investigation could proceed on more rational grounds than it has in the past. To gain experience with fungal material, work has been started on *Penicillium griseofulvum* mycelium which we can get in bulk from Imperial Chemical Industries. At a later date, when we have studied a few easily procured funguses, and when we know what sort of material to look for, work will be done on deliberately grown cultures of soil organisms.

With this organism about half the mycelial N is protein and 10-12 per cent is in the form of amino sugars behaving like glucosamine. Amino sugars are a characteristic feature of other fungal mycelia whereas they are relatively unimportant in plant residues. The identification of amino-sugars, the determination of their mode of linkage, and the study of the extraction and stability of the amino-sugar compounds under conditions similar to those in soil, is therefore an important part of our present and projected work.

Technological production of Leaf Protein

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

Work on the large scale production of leaf protein is mainly done at the Grassland Research Station, but some ancillary small scale work is done here. We have designed, and now have running a mill that deals satisfactorily with every lush agricultural leaf we have tried in it (about 10), and produces a pulp at the rate of about 1 ton (wet weight) per hour, from which liquor carrying 30-60 per cent of the leaf protein can be expressed. We have designed several continuously acting arrangements to press out the liquor from this ground mass, and the latest one, made at the National Institute of Agricultural Engineering, is reasonably satisfactory, though we see that more work is still necessary on this aspect of the problem. Heat coagulation and separation of the protein present no particular problems. The unit should be able to produce protein at a reasonable rate during the spring of 1951.

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