ROTHAMSTED RESEARCH

Report for 1939-45

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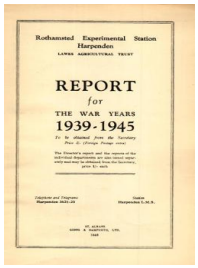

Biochemistry Department

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N. W. Pirie (1946) *Biochemistry Department ;* Report For 1939-45, pp 155 - 157 **- DOI: https://doi.org/10.23637/ERADOC-1-87**

SECTION OF BIOCHEMISTRY

By N. W. Pirie

No formal biochemical work was being carried out in Rothamsted at the beginning of 1940, but in the autumn of that year two independent biochemical laboratories were started. In October N. W. Pirie was appointed Virus Physiologist on the understanding that this title was to be interpreted practically as Biochemist, and a little later an A.R.C. unit under J. H. Quastel was set up, but has now been transferred elsewhere. The Biochemistry Section was an offshoot of the Plant Pathology Department, but as the scope of the work is being extended it has now become separate.

In 1941 E. M. Crook came on an A.R.C. grant to investigate the conditions governing the release of protein from the normal leaf. With the arrival of the electron microscope he has been more and more fully occupied with this and has now been transferred to the staff of the Plant Pathology Department. M. Holden came in 1944 and M. V. Tracey in 1945 both on A.R.C. grants to study changes that take place in normal or virus-infected leaves during the operations used in the extraction of viruses. During the second half of 1945 M. Grégoire was working here on a fellowship from the French National Centre for Scientific Research.

All the work on the extraction and fractionation of tobacco-mosaic, tomato bushy-stunt and the tobacco-necrosis viruses was carried out in this laboratory, but having been described in another section need not be set out again.

The conditions governing the release of normal protein from the leaf fibre have been studied in some detail on tobacco and more superficially on some other leaves. Fine grinding, especially in the absence of salts and under slightly alkaline conditions, leads to the separation of nearly all the protein in a soluble form. Much of this is associated with chlorophyll and lipids and is macromolecular, that is to say it can be sedimented in fields of 15,000 R.C.F. though not in fields below 2,000 R.C.F. As the work has proceeded it has become clear that fine grinding is not a simple process of dispersion, but leads also to the condensation of proteins and other leaf components. This makes the interpretation of some results both on normal leaves and on virus-infected ones doubtful. Enzymic methods of disintegration have been sought and considerable success has been achieved with the digestive fluid from snails.

It is to be expected that after maceration many leaf enzymes will be able to attack their naturally occurring substrates. The liberation of viruses on mincing the leaf may not therefore always be a purely mechanical matter, but may be associated with these enzyme actions. The demethylation of pectin has been studied, not because there is any reason to associate it with virus liberation, but because no rigid control of the pH of a leaf suspension is possible until this action has been either inhibited or allowed to go to completion. At pH 6, the normal pH of tobacco, demethylation proceeds slowly, but on raising the pH to 7 or 8 there is a very

https://doi.org/10.23637/ERADOC-1-87

great increase in the rate. This is due partly to the greater activity of the enzyme at the higher pHs and partly to the enzyme being released from the fibre in alkaline solution so that enzyme and substrate are more accessible to one another. In some leaves, notably those of the Cucurbitaceae, the sap is already sufficiently alkaline for enzymic demethylation to occur spontaneously. In these leaves also, the pH rises as successive aqueous extracts of the fibre are made. The high pH is due to the presence of calcium ions in the extracts uncompensated by an anion, and in a solution sufficiently free from other buffering material, the fourth or fifth extract for example, the pH may rise to 10. No fully satisfactory picture has yet been achieved of the mechanism of this action but some more or less plausible model systems have been made and work on its interpretation is proceeding. There are marked differences between plant species both in the total amount of pectase present and in the distribution of the enzyme between the fluids and the structure of the leaf. A few experiments have shown comparable differences between leaves of the same species grown under different manurial conditions. It would seem therefore that biochemical data compiled from plants gathered in a rather casual manner, for example in hedgerows, may be extremely misleading and that conclusions should only be based on extensive series of plants grown under a range of manurial and cultural conditions.

Plant proteases are well known, but they are generally derived either from a seed or a latex, inconclusive evidence for the presence of proteases in non-lactiferous leaves has been published. The matter is of importance because, although most plant viruses are completely resistant to attack by the usual proteases when in the native state, some are susceptible to proteolysis. Furthermore the low apparent protease activity of a system that is capable of such active nitrogen metabolism as the leaf is a matter for surprise. The unequivocal demonstration of a protease in tobacco sap is also important because of the claim that tobacco-mosaic virus has protease activity. Since the purified virus is known to combine with proteases a demonstration that healthy tobacco sap contains protease robs the claim of its validity. Trustworthy methods for the detection of dilute protease solutions have been developed, and these show that normal tobacco sap has an activity similar to that of a 0.03 gram-per-litre solution of trypsin. 75 per cent. of the enzyme that has been recognised is in the sap and it can be concentrated by precipitation with ammonium sulphate. The high dilution of the original extract and the instability of the enzyme make it unprofitable to attempt rigorous purification. The protease is not naturally fully active and a 10-fold activation is possible with reducing agents: when activated, the amount of enzyme present in the leaf is sufficient to hydrolyse 10 per cent. of the leaf protein every 24 hours. Protease activity, like pectase activity, depends on the manurial and cultural antecedents of the plant.

In 1939 work that had already been done on the antigens of *Brucella melitensis* was extended to *Br. abortus* in the hope that products with diagnostic and immunising properties could be prepared. By the autumn of 1940 the results were sufficiently

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encouraging to justify the transfer of the work to Rothamsted: it is not of course proposed that the study of an organism causing an animal disease should be a permanent feature of work in this laboratory. The fundamental hypothesis is that immunising potency depends on the size of the antigen with which an animal's protective mechanism is confronted, for foreign materials of different sizes and stabilities are dealt with by different mechanisms and not all these lead to immunity. This assumption has been borne out and fractions have been made causing a high degree of immunity in guinea-pigs. Tests on heifers are now in progress. All the animal testing has been done at Cambridge, Weybridge or Compton. The most effective antigen has a particle rather larger than those of the plant viruses studied here and it is a complex of at least two phospholipids with the N-formyl derivative of an amino-polysaccharide.

PUBLICATIONS

1. HOLDEN, MARGARET. 1945. *Acid-producing mechanisms in minced leaves.* Biochem. J., **39,,** 172-178.

The drift of pH towards the acid side observed when minced tobacco leaves are neutralised with alkali is mainly due to the enzymic demethylation of pectin when the pH is raised above that of the normal sap. The metabolic production of carbon dioxide is also in part responsible for the drift.

The liberation of methanol is more rapid in milled than in minced leaf fibre, and at pH 8 more rapid than at pH 6, whether in the presence or absence of sap.

Heating to 100°C. prevents the pH drift, and the small amount of methanol liberated at pH 8 is due to non-emzymic demethylation.

The pectase is extracted from fibre at pH 8 but not at pH 6. The pH 8 extract will demethylate boiled fibre and extract pectin.

Minced leaves of some Cucurbitaceae have a spontaneous alkaline drift when washed, which permits demethylation without the addition of alkali.

An improved method for the estimation of methanol is described.

2. HOLDEN, MARGARET. *Studies on pectase.* Biochem. J. In the press.

3. CROOK, E. M. *Extraction of nitrogen from green leaves.* Biochem. J. In the press.

4. PIRIE, N. W. *The manometric determination of formic acid.* Biochem. J. In the press.

https://doi.org/10.23637/ERADOC-1-87