

Macfarlane's⁹ recent description of an acetone insoluble *O*-alanyl ester of phosphatidyl glycerol isolated from the Gram-positive bacillus *Cl. welchii* emphasizes the complexity of these compounds and supports the hypothesis that these complexes are important metabolic constituents of the cell.

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Peroxidase Isozymes in Root Nodules of Various Leguminous Plants

MULTIPLE forms of various enzymes (isozymes) have been shown in tissues of animals¹⁻³ and plants^{4,5} and in micro-organisms⁶. The difference in electrophoretic mobility of some isozymes obtained from different species has been reported^{4,5,7}. This communication reports the existence of peroxidase isozymes which differ electrophoretically in root nodules of each of four leguminous plants. The leghæmoglobins extracted from these different nodules also differ electrophoretically.

Plants of *Galega officinalis*, *Lotus pedunculatus*, *Trifolium repens* and *Vicia sativa* were grown from seed in sterilized sand which had been inoculated with the appropriate *Rhizobia*. Nodules were collected six weeks after planting and were kept at 3° until extracted within 3 h of collecting.

Extracts were prepared by grinding 8 g of nodules in a chilled mortar with 8 ml. of 0.05 M *tris* hydrochloric acid buffer at pH 7.5. This method of extraction does not disrupt the *Rhizobia* which inhabit the nodules⁸. The slurry was centrifuged at 20,000g for 20 min and the supernatant was fractionated with ammonium sulphate at 3°. The protein precipitated between 55 and 80 per cent saturation ammonium sulphate was dissolved in 3 ml. of 0.05 M *tris* hydrochloric acid buffer at pH 7.5 and dialysed against 200 ml. of the same buffer at 3° for 18 h.

Starch-gel electrophoresis was carried out as described by Smithies⁹ in 0.05 M *tris*-citrate buffer pH 8.6. After completion of the run the gel was sliced horizontally in two. One half was stained by amido black to reveal protein bands and the other half was stained to reveal the peroxidase bands by immersing it in a solution containing 0.025 per cent benzidine, 1 per cent hydrogen peroxide and 5 per cent acetic acid in 50 per cent aqueous ethanol. The parts of the starch gel containing peroxidases showed a blue coloration. Leghæmoglobins, which could be identified as brown bands prior to staining, gave the peroxidase reaction when stained with the benzidine reagent.

In both Figs. 1 and 2 leghæmoglobins are marked by the letters A and B. It will be seen that the two leghæmoglobins in the nodules of the four plants vary in electrophoretic mobility. The slowest moving bands are those of *Trifolium repens* and *Galega officinalis* followed by those of *Vicia sativa* and *Lotus pedunculatus*. The

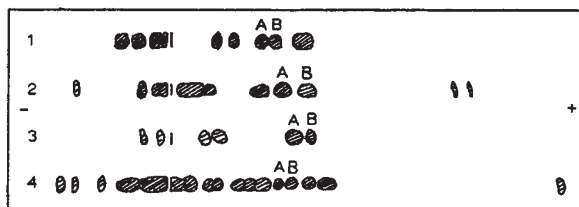


Fig. 1. Electrophoretic pattern of peroxidases in root nodule extracts. 1, *Trifolium repens*; 2, *Vicia sativa*; 3, *Lotus pedunculatus*; 4, *Galega officinalis*

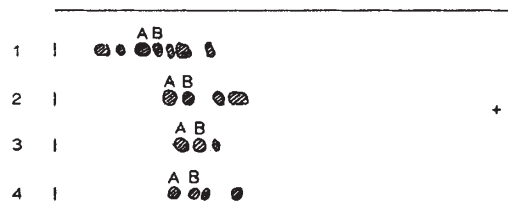


Fig. 2. Electrophoretic pattern of proteins in root nodule extracts. 1, *Trifolium repens*; 2, *Vicia sativa*; 3, *Lotus pedunculatus*; 4, *Galega officinalis*

difference in electrophoretic mobilities between leghæmoglobins of root nodules of different plant genera has a counterpart in animal kingdom, where there are differences between electrophoretic mobilities of hæmoglobins of various species of vertebrates¹⁰.

The zymograms of peroxidases in root nodules (Fig. 1) show a characteristic pattern for each of the plants used in this work. The most outstanding differences between the peroxidase patterns are the fast sharp bands moving towards the anode in nodule extracts of both *Galega officinalis* and *Vicia sativa* which are not present in extracts of nodules of the other two plants. Although most of the peroxidase bands showed intense enzymatic reaction they did not contain enough protein to stain with amido black. In the gels that were stained with amido black no bands were observed on the cathode side of the origin (Fig. 2).

The specific role of each of the peroxidase isozymes in root nodules and the biological significance of the difference in the peroxidase pattern in nodules of different leguminous plants remain to be established.

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PHYSIOLOGY

Storage of Insecticides in French People

STORAGE of DDT in people without occupational exposure to the compound reflects their dietary intake¹. Measurement of storage in a random sample of the population of the country may be used as an index of residues of other pesticides as well as those of DDT in the total food supply. We were fortunate in obtaining samples of adipose tissue from ten French people. Even though the number is small, the results are of interest because they are the first from France and because they permit a comparison with results from other countries.