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Phenolic Metabolism in Potato Tuber Tissue after Infection with *Phytophthora infestans*

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Phenolic compounds accumulate in potato tissue after inoculation with *Phytophthora infestans*. Chlorogenic acid, scopolein (Hughes & Swain, 1960) and a lignin-like polymer (Friend & Knee, 1969) have been found in infected susceptible tubers (var. Majestic), whereas suspended tissue cultures of R₁-resistant potatoes (var. Orion) accumulate salicylic acid, vanillic acid and *p*-hydroxybenzoic acid after inoculation with an incompatible race of the fungus (Robertson *et al.* 1968).

We compared the changes in phenolic compounds and of phenylalanine ammonia-lyase activity of tubers of susceptible and resistant varieties after inoculation with a sporangial suspension of *P. infestans*. There was a large increase in chlorogenic acid concentration in both inoculated and control discs of Orion (resistant) compared with similarly treated Majestic (susceptible) tubers. Phenylalanine ammonia-lyase activity also increased more rapidly in Orion than in Majestic tubers, and this higher activity was maintained. There was a greater accumulation of a lignin-like polymer in both sets of inoculated tubers; the increase in Orion was more than in Majestic tubers.

The changes in lignification in relation to the spread of the fungus across the surface of tuber slices from a central inoculation site were also examined. In Majestic tubers the infected zone was 50 mm in diameter after 140 h, but in Orion tubers it did not extend beyond 15 mm, a distance reached in 40 h. In Orion tubers maximum lignification was after 70 h near the centre of the infected area, whereas in Majestic tubers lignification was spread over a wider area and the highest values were obtained in the outer portion of the infected area after 140 h. The values of phenylalanine ammonia-lyase activity followed a similar pattern.

Lignification may well be involved in the restriction of the spread of the fungus in Orion tubers; similar suggestions have been made for other host-parasite combinations (Hijwegen, 1963) and the extent of lignification could be controlled by the activity of phenylalanine ammonia-lyase. Lignin could act as a barrier by modifying the galactan-

containing polymers in the cell wall so that they can no longer be hydrolysed by the *P. infestans* galactanase (Knee & Friend, 1970).

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The Effect of γ -Irradiation on the Amino Acid Content of Proteins

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The technology of γ -irradiation has been developed and applied to foods to prevent loss by insect depredation and by bacterial and fungal attack. The hazards of radiation have led to intensive research applied to the task of showing that irradiated foods are safe to eat. Far less effort has gone into a study of the effect of radiation on the nutrients contained in the foods. There is evidence that radiation can cause change or destruction of free amino acids. Amino acids in proteins are also susceptible, but reports vary as to the nature and extent of the damage. The discrepancies in the literature may result from variations in the conditions of irradiation, such as temperature, oxygen partial pressure, water content, dose and dose rate.

We have investigated the amino acid content of accelerated-freeze-dried egg (Affined Foods Ltd., High Wycombe, Bucks., U.K.) and two batches of wheat gluten (BDH Chemicals Ltd., Poole, Dorset, U.K.). The moisture contents were 1.6, 6 and 10% respectively. These were distributed into cellophane-polythene laminate bags, sealed under 96% N₂, and irradiated at room temperature at dose rates of approx. 1.5 Mrd/h in the Spent Fuel Rod Assembly, Atomic Energy Research Establishment, Harwell, Berks, U.K., for total doses of 0.0, 0.5, 2.5 and 5.0 Mrd, and, for the second batch of gluten, 10 Mrd. After irradiation and transport to our laboratory, the samples were stored at -20°C until analysed by hydrolysis and ion-exchange chromatography. In egg the only amino acids significantly damaged were methionine and tryptophan. In gluten tryptophan in both batches, and threonine in batch 1 and methionine in batch 2 were significantly diminished. An apparent effect on aspartic acid, serine, glutamic acid, glycine, leucine, tyrosine

and histidine observed in batch 2 is attributable to the increased recovery of amino acids from the samples irradiated at 0.5 Mrd compared to the unirradiated control samples and not to damage caused by the higher levels of irradiation.

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Thiamin Balance in the Sheep

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The development of an automatic device for sampling digesta leaving the abomasum of the sheep (Axford, Evans & Offer, 1971) has made it possible to study the thiamin balance across the stomach of a 37 kg Welsh Mountain wether fitted with re-entrant duodenal cannulae. Previous studies (e.g. Kon & Porter, 1953; Phillipson & Reid, 1957) have been limited to concentrations, as simultaneous measurements of flow rates were not reported.

This sheep was fed on a constant pelleted ration supplying 2 mg of thiamin daily, supplemented by the intra-ruminal infusion of increments of thiamin. At the basal level the daily outflow of thiamin equalled the inflow. Increases in the thiamin intake by infusion produced smaller increases in the rate of passage of thiamin from the abomasum, indicating the absorption or destruction of about half the additional thiamin. Although the rate of thiamin outflow from the abomasum was never found to be more than the intake, extrapolation of the regression line relating passage of thiamin from the abomasum to total daily intake indicated an endogenous synthesis of about 1 mg of thiamin at zero intake. The concentration of thiamin in the duodenal contents increased proportionately with intake, which agrees with the previous studies referred to. Thiamin determinations were also carried out on representative samples of faeces and urine and indicated that excretion of thiamin by these routes was virtually unaffected by varying the intake.

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Growth of *Pseudomonas reptilivora* on Glycine

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We are interested in C₁-unit biogenesis in micro-organisms growing on carbon sources from which glycine can be derived by metabolic pathways not involving serine. As part of this study we have been investigating the growth of *Pseudomonas reptilivora* (N.C. 8906) on a simple inorganic salts medium containing either glycine or an N-methylated derivative thereof as sole carbon and energy source.

Extracts of glycine-grown cells have been found to possess high activities, as compared with extracts of succinate-grown cells, of serine dehydratase and of an enzyme system (the 'glycine enzyme') catalysing the pyridoxal phosphate- and tetrahydropteroylglutamate-dependent oxidation of glycine to CO₂, ammonia and N⁵N¹⁰-methylene-tetrahydropteroylglutamate (e.g. see Sagers & Gunsalus, 1961; Pitts & Crosbie, 1962; Bull & Woods, 1963). Serine hydroxymethylase activities are also increased, although only about fourfold, during growth on glycine. Glycine oxidase and glyoxylate carboxylase are not detectable and malate synthase activity is uniformly low in extracts of glycine-grown cells and of succinate-grown cells.

It is concluded that the pathway of utilization of glycine by *Ps. reptilivora* involving conversion into serine and thence into pyruvate is analogous to that reported by Jones & Bridgeland (1966) for *Arthrobacter globiformis* and different from the pathway in *Pseudomonas A* (*Ps. acidovorans*) involving glyoxylate and tartronic semialdehyde (Callely, Trudgill & Dagley, 1961).

The observation that the methylene carbon of [2-¹⁴C]glycine is utilized as the sole quantitative source of C₁ residues in thymine (methyl group), serine (C-3) and purines (C-2) is consistent with this conclusion.

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