The Metabolism of Fluoroacetate by Plants

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Fluoroacetate is highly toxic to most animals because of its metabolism to fluorocitrate (Peters, 1963), but it is relatively non-toxic to plants even though they use the tricarboxylic acid cycle. Recently fluoroacetate and fluorocitrate have been isolated from forage crops that have grown in an environment rich in fluoride (Lovelace, Miller & Welkie, 1968) and it would be of interest to know more about the metabolism of compounds containing a carbon–fluorine bond, by plants.

Lettuces were each placed in a closed desiccator with their roots immersed in water. Air was bubbled through the water and the effluent gas from the desiccator was dried with calcium chloride and then bubbled through 2-methoxyethanol–ethanolamine (2:1, v/v) (Jeffay & Alvarez, 1961) to trap carbon dioxide. The desiccator was constantly illuminated. Plants were incubated in this apparatus in one of three ways: (1) for 43hr. with sodium [1-^{14}C]acetate (1mg./kg. wet wt.), (2) for 21hr. with sodium fluoroacetate (50mg./kg. wet wt.) followed by 43hr. with sodium [1-^{14}C]acetate (1mg./kg. wet wt.), or (3) for 43hr. with sodium fluoro[1-^{14}C]acetate (50mg./kg. wet wt.) (Ward & Huskisson, 1966).

In the [^{14}C]acetate-treated lettuces, 15–20% of the radioactivity was expired as carbon dioxide, and this was unaffected by the presence of non-radioactive fluoroacetate. In the fluoro[^{14}C]-acetate-treated plants, only 0·5% of the radioactivity appeared in the carbon dioxide. Citrate concentrations in fluoroacetate-treated plants were about 28 μmoles/g. dry wt., almost double those of the untreated plants (15 μmoles/g. dry wt.).

Plants treated with fluoro[^{14}C]acetate were examined to ascertain the distribution of radioactivity. All the radioactivity could be extracted by ethanol–water mixtures (Canvin & Beevers, 1961) and fractionation on Sephadex G-25 followed by cation- and anion-exchange columns showed that it was associated with low-molecular-weight acidic material. Continuous ethereal extraction removed about half the radioactivity in the form of fluoroacetate and fluorocitrate, the former greatly predominating. No trace of radioactivity was found in the fatty acids. The concentration of fluorocitrate was 0·15 μmole/g. dry wt. of plant. It was detected by gas chromatography and its identity was confirmed by silicic acid chromatography (Ward & Peters, 1961) and paper chromatography. The remaining half of the radioactivity unextractable by ether was associated with one or more compounds highly acidic in nature and insoluble in organic solvents. Attempts to isolate them by gas–liquid chromatography as methyl esters or trimethylsilyl ethers were unsuccessful. After purification on a cellulose column (Isherwood & Barrett, 1967) the radioactive compounds separated into two discrete spots on two-dimensional paper chromatography. The isolation of quantities of these compounds is now proceeding.

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An Organically Combined Fluorine Compound in Bone

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It is of general interest to know whether there can be present in bone organically combined fluoride in addition to inorganic fluoride. We were led to examine this question as a consequence of work on plant homogenates (Peters & Shorthouse, 1967), work on lettuce (P. F. V. Ward, unpublished work), work reported by Little *et al.* (in preparation), and the finding of Cheng, Ming-Ho, Miller and Wilkie (1968) that ordinary forage plants can convert fluoride into fluorocitrate.

We have now found that extracts of some fluorosed bones from cattle contain small amounts of fluorocitric acid, which have been identified by their capacity for inhibiting aconitate hydratase (aconitase), and by gas–liquid chromatography. We are investigating whether this is present in the pasture. Though it is well known that fluorocitrate formed from fluoroacetate inside mitochondria is very toxic, virtually nothing is known about its behaviour when ingested.

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