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 43 hr. with sodium [^{14}C]acetate (1 mg./kg. wet wt.), (2) for 21 hr. with sodium fluoroacetate (50 mg./kg. wet wt.) followed by 43 hr. with sodium [^{14}C]acetate (1 mg./kg. wet wt.), or (3) for 43 hr. with sodium fluoro[^{14}C]acetate (50 mg./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 $\mu\text{moles/g. dry wt.}$, almost double those of the untreated plants (15 $\mu\text{moles/g. dry wt.}$).

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 $\mu\text{mole/g. dry wt. of plant}$. 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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Stimulation of Incorporation of Precursors into Protein and Ribonucleic Acid of Rat Liver Slices by Bovine Growth Hormone *in vitro*

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Stimulatory effects of growth hormone on RNA and protein synthesis can be observed when it is added to the medium perfusing rat liver (Jefferson & Korner, 1967), but Jackson & Sells (1967) reported that growth hormone *in vitro* had no effect on RNA labelling in liver slices. The concentration of amino acids present during perfusion affects the response to growth hormone, so it seemed possible that the absence of an effect of growth hormone on liver slices was due to the absence of amino acids. This point was investigated and the effects of growth hormone on amino acid incorporation into protein in liver slices were also studied.

Liver slices from male Sprague-Dawley rats, weighing 100–120g., were incubated for 1 hr. in Krebs-Ringer bicarbonate medium containing 3% bovine serum albumin, 8mm-glucose and a mixture of twenty amino acids in the proportions in which they occur in rat serum (Schimassek & Gerok, 1965), with a constant specific radioactivity of [^{14}C]leucine and 1 μC of [^3H]orotic acid/ml. The slices were homogenized in 0.9% NaCl at the end of the incubation period and after precipitation with 0.5M-HClO₄, the protein was freed of amino acids, RNA and lipids, and radioactivity was measured by the procedure of Munro, Jackson & Korner (1964). When labelling of RNA was studied, the homogenate was treated with 20% (w/v) trichloroacetic acid and RNA was extracted and counted as described by Jefferson & Korner (1967).

It was found that optimum amino acid incorporation into protein occurred when the concentration of amino acids in the medium was six times that in rat plasma, but that the labelling of RNA was virtually unaffected by changes in amino acid concentration. At six times plasma concentration of amino acids, addition of growth hormone at a concentration as low as 10ng./ml. stimulated incorporation of amino acids into protein by 30% and of labelled precursor

into RNA by 27%. No effect of the hormone on labelling of protein *in vitro* was observed when the amino acid concentration was at the normal plasma value. Preliminary observations suggest that RNA labelling is stimulated by the hormone under the latter conditions but not when amino acids are absent entirely.

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Studies on Drug-Metabolizing Activity in Rats Pretreated with Phenobarbitone and Compounds Related to Emetine

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Protein synthesis at the liver microsomal level *in vitro* can be stimulated maximally in incubations of preparations from female albino rats pretreated with sodium phenobarbitone at 315 $\mu\text{moles/kg.}$ daily for 3 days (Kato, Jondorf, Loeb, Ben & Gelboin, 1966), or with (–)-emetine dihydrochloride (Jondorf & Szapary, 1968) or ($\pm$)-2,3-dehydroemetine dihydrochloride (Jondorf, Drassner, Johnson & Miller, 1969) at 18 $\mu\text{moles/kg.}$ for 1 day.

Although the microsome-specific stimulation of protein synthesis in phenobarbitone-pretreated animals is also associated with stimulation of microsomal drug-metabolizing activity (Conney, 1967), this is not the case in experiments with microsomal material from rats treated with emetine or 2,3-dehydroemetine. These compounds inhibit drug metabolism *in vitro* (Jondorf, Johnson & Drassner, 1969) measured by liver microsomal *N*-demethylation of aminopyrine (Jondorf, Simon & Avnimelech, 1966) and azo-reduction of neoprontosil (Hernandez, Gillette & Mazel, 1967).

It therefore seemed worth while to test whether the co-administration of phenobarbitone and emetine or 2,3-dehydroemetine would lead to diminished drug metabolizing effects. Two injection schedules were devised, to differentiate between effects of emetine and 2,3-dehydroemetine on already existing, phenobarbitone-induced stimulation and effects on incipient induction. In the