Biochemical Transformations of Pesticides by Soil Micro-organisms

By W. C. EVANS (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor*)

There have been two main approaches employed to elucidate the fate of chemicals used in agricultural practice, when sprayed on to crops or added to the soil:

In the case of physiologically active substances, bioassays and/or very sensitive physicochemical techniques including tracer, chromatographic and spectroscopic procedures, applied to the materials treated *in situ*.

If pesticide disappearance is caused by biological agents, as is often the case, isolation of competent micro-organisms from the soil milieu by elective culture, followed by a conventional biochemical attack on the metabolic pathway in pure culture.

Both approaches have their limitations, in many ways they are complementary, and both are essential if we are to understand the processes in detail.

Several factors may influence the persistence of a pesticide within the soil ecosystem:

Inherent physicochemical properties of the molecule—solubility volatility, stability in the environment etc.

Reaction of the chemical with the soil colloids (both inorganic and organic).

Failure of the particular chemical structure to cause the induction of the necessary enzymes capable of coping with it, by the micro-flora, in that environment.

Considerable progress has been made towards elucidating biochemical pathways of metabolism of many important pesticides, in the course of which several interesting enzymes have been revealed; some of these will be discussed.

Other important considerations are the effects of these foreign chemicals on the fundamental biochemical cycles normally occurring in soils.

Influence of γ -Radiation on some of the Biochemical Properties of Soil

By P. A. CAWSE (*United Kingdom Atomic Energy Authority, Wantage Research Laboratory, Wantage, Berks.*)

Since 1950, sixty research publications have covered various aspects of soil response to radiation. They are mainly concerned with sterilization and its effects on humus and enzymes, plant growth in irradiated soil, and radiosensitivity of the microflora.

Few organisms survive γ doses of 2–4 Mrads, but

humus is relatively unaffected and the activity of phosphatase, urease and some other enzymes remains intact. Increased plant growth in soil treated with 0.005–4 Mrads is mainly attributed to death and lysis of susceptible organisms followed by nitrogen mineralization. In many soils, the ammonium is rapidly oxidized by the surviving nitrifiers, and nitrate reaches a maximum between 0.2 and 0.8 Mrad; perfusion studies suggest that radiation prevents proliferation without affecting oxidation processes. Nitrification is gradually inhibited from 0.8 to 3 Mrads, with a corresponding increase in ammonium. In two organic calcareous soils treated over the range 0.6–2.5 Mrads, nitrite appears to form by reduction of nitrate with maximum accumulation at the lower dose region.

There are considerable differences in lethality of radiation towards the microflora, both between and within major groups such as fungi and bacteria. Microbial oxidation of sulphur shows a radiation response curve with two distinct slopes, indicating that separate organisms are involved. There is evidence for harmful and stimulatory effects on nitrogen fixation, and natural populations of *Azotobacter* are more radioresistant than laboratory-grown bacteria.

The Rhizosphere and its Influence on Ion Uptake by Plants from Soils

By D. A. BARBER (*Agricultural Research Council Radiobiological Laboratory, Letcombe Regis, Wantage, Berks.*)

The roots of plants growing in soil are surrounded by very large numbers of micro-organisms, which vary greatly in their ability to either increase or decrease the availability of inorganic nutrients. Similarly, there is an extensive, though more limited, microflora associated with plants grown in water culture.

In most studies of the absorption of inorganic nutrients by plants, whether in soil or water culture, little consideration has been given to the possible effects of the attendant micro-organisms.

Methods were therefore devised that enabled the absorption and distribution of phosphate in young barley plants to be compared under sterile and non-sterile conditions. It has been shown in water culture that micro-organisms present on roots at ambient laboratory concentrations rapidly accumulate phosphate, causing its rate of incorporation into nucleic acids and other complex organic compounds by the plant to be greatly overestimated at concentrations below about 1.0 p.p.m. of P. This immobilization of phosphate lowers the amount actually available to the root cells and causes less

to be transferred to the shoots. The extent of this microbial participation in the apparent absorption of phosphate by the plants is influenced by several factors: first, it is of importance only at low concentrations; secondly, because of the known sensitivity of bacteria to hydrogen ion concentra-

tion, it increases with increasing pH; and thirdly, it is affected by the exudation of organic substances from the root.

These findings will be discussed in relation to the possible influence of the rhizosphere flora on the absorption of inorganic nutrients by plants.

COMMUNICATIONS

Threonine Deaminase and Acetolactate Synthetase in *Anabaena variabilis*

By W. HOOD and N. G. CARR (*Department of Biochemistry, University of Liverpool*)

Recent work has indicated that species of blue-green algae hitherto considered to be strict autotrophs will incorporate and metabolize carbon compounds other than carbon dioxide; ^{14}C -labelled sugars, carboxylic acids and amino acids were assimilated into *Anabaena variabilis* and *Anacystis nidulans*. However, Pearce & Carr (1967) have found no evidence of repression or de-repression of enzymes concerned in acetate metabolism after growth in the presence of this substrate. This communication describes the initial attempts to estimate the extent of metabolic control by either repression or feed-back inhibition in amino acid biosynthesis. This has been only briefly examined in the blue-green algae: Hoare & Hoare (1966) demonstrated feed-back control in arginine formation.

Anabaena variabilis was grown (Pearce & Carr, 1967) autotrophically and in the presence (100 mg./l.) of L-valine or L-valine, L-leucine and L-isoleucine; L- ^{14}C valine was shown to be assimilated by the significant uptake of ^{14}C into cell material during growth. Cell-free extracts from each type of culture were prepared by ultrasonic treatment and the activities of enzymes concerned in branched-chain amino acid biosynthesis were examined. Acetolactate formation from pyruvate was measured by conversion into acetoin and spectrophotometric determination of this product (Westerfield, 1945). Acetolactate synthetase activity had a broad pH range with two optima, indicative of two isoenzymes, as found in *Escherichia coli* (Radhakrishnan & Snell, 1960). Variations in the growth conditions quoted above did not alter the specific activities of this enzyme in extracts at any pH value, but valine exerted 50% inhibition on activity in extracts assayed at pH 8.0. A lack of repression was also observed when threonine deaminase was examined in extracts from organisms grown autotrophically (0.17 $\mu\text{mole/mg. of protein/hr.}$) and in the presence of isoleucine (0.16 $\mu\text{mole/mg./hr.}$) or valine, leucine and iso-

leucine (0.19 $\mu\text{mole/mg./hr.}$). In contrast with the failure to exhibit enzyme repression, threonine deaminase was competitively inhibited by its end product, isoleucine, and this action was relieved by valine. In this respect threonine deaminase in blue-green algae is similar to that in other organisms.

The lack of enzymic repression in *Anabaena variabilis* contrasts with results obtained with other procaryotic organisms and indeed with *Anacystis nidulans*, preliminary results with which indicate slight repression of acetolactate synthetase by growth in the presence of valine.

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Reduced Nicotinamide-Adenine Dinucleotide Phosphate Oxidase in the Autotrophic Blue-Green Alga *Anabaena variabilis*

By C. K. LEACH and N. G. CARR (*Department of Biochemistry, University of Liverpool*)

The observation that there is significant assimilation of reduced ^{14}C -labelled carbon compounds by strict autotrophs has renewed interest in the causative reasons of obligate autotrophic metabolism. The earlier view that such organisms were generally impermeable to organic compounds is now unlikely to be correct. An incomplete tricarboxylic acid cycle has been observed by several groups and its role in autotrophy discussed. Smith, London & Stanier (1967) suggest that the absence of α -oxoglutarate dehydrogenase and, more crucially, NADH oxidase, are the essential features of obligate autotrophy in both blue-green algae and the chemosynthetic bacteria, and have suggested certain evolutionary implications of these deletions.

This communication reports that in extracts of several blue-green algae an NADPH oxidase is