PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 504th Meeting of the Society was held at the University College of Wales, Penglais, Aberystwyth, on Wednesday and Thursday, 1 and 2 April 1970, when the following papers were presented:

COLLOQUIUM ON 'THE INTERACTION OF PESTICIDES AND HERBICIDES WITH BIOCHEMICAL SYSTEMS'

The Mode of Action of Paraquat and Diquat

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Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride) and diquat (1,1'-ethylene-2,2'-bipyridylium dibromide) are widely used in formulations as quick acting desiccant herbicides. They are the active components of the herbicides Reglone and Gramoxone, marketed by Plant Protection Ltd., Bracknell, Berks., U.K. Initial work on the mode of action of diquat by Mees (1960) indicated that oxygen and light were essential for the kill of green plants. Zweig, Shavit & Avron (1965) further elucidated the light requirement in experiments with isolated chloroplasts. They found that diquat caused a deviation of electron flow from photosystem I, leading to an inhibition of NADP⁺ reduction and the production of a reduced diquat radical. A possible requirement for oxygen was established by Davenport (1963), who found that the reduced diquat free radical was reoxidized with the production of hydrogen peroxide.

Further evidence for the production of hydrogen peroxide has been obtained in experiments with heated chloroplasts, which have no photosystem II activity, and no requirement for dichlorophenol-indophenol (Davenport & Dodge, 1969) to mediate electron flow from ascorbate. In the presence of paraquat illuminated chloroplasts showed an oxygen uptake, part of which was released by the addition of catalase in the dark. However, if catalase and ethanol were included in the chamber during illumination, acting as a trap for hydrogen peroxide, no oxygen evolution was obtained on the addition of further catalase.

By using flax (*Linum usitatissimum* var. Red Wing) cotyledon leaves floated on a 0.1 mM solution of paraquat or diquat under continuous illumination, the sequence of some of the events occurring during the kill was followed.

Photosystem II activity, as measured by ferri-

cyanide reduction of isolated chloroplasts, was inoperative after 20 h of treatment in the light. At this stage there was little visible evidence of herbicidal activity. As photosystem I, with which the bipyridyls interact, is dependent on photosystem II *in vivo*, we concluded that there was no further production of primary toxic products after 20 h of continuous illumination. When leaves were illuminated for 6 h and then put into the dark, photosystem II inactivation continued, but at a lower rate. If a dark period was commenced after 15 h of continuous illumination, the light and dark inactivations from this point were identical. With chlorophyll contents, however, a lower rate of breakdown was observed in the dark, even after 20 h of continuous illumination. This increased breakdown was probably due to photo-oxidation. The breakdown of chlorophyll increased rapidly in continuous illumination after 20 h, with a more rapid breakdown of chlorophyll *a* than of chlorophyll *b*.

Malondialdehyde, a breakdown product of polyunsaturated fatty acid hydroperoxides (Kohn & Liversedge 1944; Patton & Kurtz, 1951), was measured in treated leaves as an indication of lipid peroxidation. Production began after 6 h of herbicide treatment, and continued to increase during the next 48 h. The loss of galactosyl lipids indicated that there could well be a breakdown of cell membranes, and electron micrographs showed a disruption of the tonoplast in some cells after 6 h. There was an increase in membrane permeability at this time, as indicated by an increased efflux of K⁺ from treated leaves (cf. Eilam, 1965). Soluble protein contents were shown to decrease. Electron micrographs obtained after up to 30 h of treatment showed a massive increase in the number and size of osmiophilic globules (Lichtenthaler, 1968) and also a considerable change in the appearance of chloroplast nucleic acids.

It is considered that the breaking of the tonoplast would lead to a rapid change in osmotic equilibrium, which would have considerable effects on the cytoplasmic organelles. If the vacuole is classed as a lysosome (Matile & Moor, 1968), the release of hydrolytic enzymes, including proteases, nucleases

and phosphatases, would have a dramatic effect on cell components.

Although the pattern of breakdown of many cell constituents is similar to that found during natural senescence but on a shorter time-scale, significant differences in comparative times of changes in membrane permeability and photosynthetic activity (Baldwin, Dodge & Harris, 1968; Drury & Park, 1968) indicate that the herbicidal activity is a fundamentally different process.

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The Biochemistry of Warfarin Resistance in the Rat

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Warfarin-resistant rats were first reported on farms in Scotland (Boyle, 1960) and have since appeared in Denmark (Lund, 1964) and in areas of Montgomeryshire and Shropshire (Drummond, 1966). The resistance is inherited as a single autosomal dominant gene, which is linked to coat colour (Greaves & Ayres, 1967, 1969).

Whereas in normal rats ingestion of warfarin results in the blood's becoming incoagulable, in resistant animals blood clotting times are only slightly prolonged. It is now generally accepted that the sole function of vitamin K in animals is to maintain the normal synthesis of clotting factors II, VII, IX and X; the effect of warfarin is to decrease the concentrations of these factors in the plasma. Warfarin has also been shown to inhibit the production of factor VII *in vitro* by rat liver slices (Pool & Borchgrevink, 1964; Lowenthal & Birnbaum, 1969).

The distribution of radioactivity in rats after the intraperitoneal administration of [^{14}C]warfarin has been followed (Link, Berg & Barker, 1965) and

the metabolites in the urine identified (Barker, Hermodson & Link, 1969). In addition to unchanged warfarin, these comprise the 6-, 7-, 8- and 4'-hydroxywarfarin, 7-hydroxywarfarin glucuronide and an intramolecular cyclization product, 2-methyl-4-phenyl-3,4-dihydropyrano[3,2-c][1]-benzopyran-5-one.

The present comparisons of various aspects of warfarin metabolism in normal and warfarin-resistant rats were facilitated by the synthesis of the following radioactively labelled warfarin species: [^{14}C]warfarin (0.80 mCi/mmol), [α - ^{14}C]warfarin (4.0 mCi/mmol), [^{14}C]warfarin (0.87 mCi/mmol) and warfarin generally labelled with tritium in the aromatic moiety of the coumarin nucleus (5.4 mCi/mmol). The total radioactivity in urine and faeces and the specific radioactivities of plasma, liver and kidneys of both types of rat were determined at intervals after single oral doses of warfarin varying from 0.2 to 100 mg/kg. Extracts of urine, plasma and liver were subjected to t.l.c. and the metabolites were compared by radioautography and by the use of spray reagents. Quantitative results from resistant animals were comparable with normal values, although the latter varied considerably from animal to animal. No significant differences in the nature and relative amounts of warfarin metabolites in plasma, liver or urine could be found by the methods employed. These initial observations, which suggested that the rate and mode of warfarin detoxication after a single dose are the same in normal and resistant rats, confirmed those of Pool, O'Reilly, Schneiderman & Alexander (1968) and Hermodson, Suttie & Link (1969).

The LD₅₀ for warfarin administered on a multiple-dose basis, however, is much lower than the acute LD₅₀ (Krieger, 1949; Hayes & Gaines, 1950; Berg, 1964; Pyörälä, 1968), and it is on a multiple-dose basis that resistant rats are selected in the field (Drummond & Wilson, 1968). Three resistant and three normal rats were each given four daily oral doses (0.2 mg/kg) of [α - ^{14}C]warfarin. Samples of plasma, urine and faeces were collected 12 h after each dose and assayed for radioactivity; 84 h after the initial dose the average plasma concentration of radioactivity was 1.65% of a single dose for the normal and 1.49% for the resistant rats. Urinary excretion accounted for 27.3% of the radioactivity of the total dose in normal and 26.5% in resistant animals. However, whereas 13.5% of the total dose appeared in the faeces of normal rats, the corresponding value for the resistant group was 24.3%. These results do not completely exclude differences between the mechanisms of warfarin detoxication in the two types of rat. Each of the metabolites of warfarin in the urine accounts for at least 5% of the administered dose (Barker *et al.* 1969) and the present work shows the amount of these metabolites