General Discussion—Session III

NEAL: I wish to discuss the mechanism of the metabolism of dialkyl aryl phosphorothioates by the mixed-function oxidase enzymes. Although, for the particular reaction I shall discuss, the electron-withdrawing group has been shown to be a substituted phenol, the mechanism should be independent of the chemical structure of this group.

The attacking oxygen species is considered to be the oxygen atom in the singlet state. This singlet oxygen atom would be a highly reactive electrophilic species, and we propose that it adds across the electron-rich P=S double bond to form a cyclic intermediate. This intermediate then undergoes an electronic rearrangement, leading to the loss of the sulfur and giving the oxygen analogue of the phosphorothioate. We believe the sulfur leaves in the atomic state, a highly reactive electrophilic species. Using ^{35}S-parathion we found that a substantial portion of the sulfur released in this reaction becomes covalently bound to macromolecules. We are now in the process of examining the nature of this covalent bond.

In the formation of the dialkyl phosphorothioic acid, we propose the same cyclic intermediate as in the reaction leading to the formation of the oxygen analogue. This intermediate then undergoes a rearrangement, leading to the loss of the aromatic group. The positively charged cyclic phosphorus–oxygen–sulfur intermediate is then decomposed, first by an attack by water on the electrophilic phosphorus, and then by the loss of one of the hydrogen atoms of water as a proton and by a cyclic rearrangement leading to the loss of the original attacking oxygen atom.

We have $^{18}O_2$ studies under way to support or refute this mechanism. If the reaction were carried out in an atmosphere enriched with $^{18}O_2$, one would expect the oxygen analogue to be labelled with ^{18}O, but the diethyl phosphorothioate would not be so labelled. On the other hand, if the reaction were carried out in ^{18}O water the diethyl phosphorothioic acid should be labelled but the oxygen analogue would not. I was pleased to find out that Dr Yamamoto has discovered precisely this pattern of ^{18}O labelling. It seems, therefore, that these proposed mechanisms may have some validity.

Dealkylations seen with these compounds could be explained by an insertion reaction of the singlet oxygen atom with a carbon-hydrogen bond on the α-carbon of one of the alkyl groups, followed by a rearrangement of the resulting substituted acetal, leading to the loss of the alkyl group.

DAUTERMAN: Reactions with microsomal mixed-function oxidases consist mostly of hydroxylations.

WILKINSON: Although singlet oxygen could possibly be one active oxygen species affecting the oxidation of phosphorothioates, it is unlikely that this species is the one associated with microsomal cytochrome P-450, which is responsible for the mixed-function oxidation of lipophilic drugs and insecticides. The nature of the active oxygen species at P-450 has not yet been elucidated, though it has been suggested that it is either $\cdot OH$ or $\cdot O_2H$.

YAMAMOTO: Work on the metabolism of Dyphonate† by liver microsomal enzymes fortified with NADPH in the presence of either $^{18}O_2$ or $H_2{}^{18}O$ indicates that dyphoxon receives its " oxon " oxygen exclusively from the air, and that the water present is the sole source of the oxygen incorporated into the thiophosphonic acid. The phosphonic acid metabolite gets oxygen from the air and from the water. Thus, it is possible that the phosphonic acid is formed by hydrolysis of dyphoxon. The results indicate that an " activated intermediate " is formed initially, which in turn decomposes to form the oxon on the one hand and the phosphonothioic acid on the other. The intermediate is commonly conceived to be a three-membered ring including P, S, and O or its equivalent resonance form. The splitting of other P=O compounds with the microsome +NADPH+O_2 system might also involve such an intermediate.

FUKUTO: We are taking a different approach in attempting to elucidate the mechanism of the P=S to P=O reaction. In the mechanism proposed by Dr Neal, it is likely that retention of configuration of the phosphorus atom would be obtained. On the other hand, if the reaction should proceed through hydroxyl radical attack, one might visualize a trigonal pyramidal intermediate, which should lose sulfur with inversion of configuration. We are at present preparing optically active thionophosphates and will oxidize them by the microsomal systems. By determining whether inversion or retention of phosphorus configuration occurs, we should be able to shed light on the mechanism of this reaction.

† Names against which this symbol appears are identified in the Glossary on pages 445-446