Cytochrome *P*-450 is a two-electron oxidizing agent when combined with oxygen and linked to NADH, although the overall reaction involves four electrons, two from the NADH. The steps of

the reaction could well involve $\left[\text{RS}^- \text{Fe(II)O}_2\text{-XH} \right]$

where XH is the substrate, $\left[\text{RS}^\cdot \text{Fe(III)O}_2^{2-}\text{-XH} \right]$,

and $\left[\text{RS}^\cdot \text{Fe(III) + XOH + H}_2\text{O} \right]$, before the last com-

plex is reduced back to $\left[\text{RS}^- \text{Fe(II)} \right]$. The very low redox potential of cytochrome *P*-450 is consistent with an anionic group such as a thiol as the proximal group to the haem.

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The Metabolism of some Substituted Benzofuran β -Adrenergic Blocking Agents

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From a number of substituted benzofurans with β -adrenoceptive blocking properties, 2-(1-hydroxy-2-isopropylaminoethyl)-6,7-dimethylbenzofuran (Ro 3-3528) was selected for clinical trial, and its metabolism studied in the rat, mouse, dog and man.

The principal metabolite in all species was 6-carboxy-2-(1-hydroxy-2-isopropylaminoethyl)-7-methylbenzofuran isolated as its methyl ester and identified by n.m.r. and mass-spectral measurements. In man this compound accounted for 90% of the urinary radioactivity. From urine of rats treated with Ro 3-3528, 6,7-dimethylbenzofuran-2-ylglycolic acid and the corresponding glyoxylic acid and carboxylic acid were identified on the basis of mass-spectral determination, chemical properties and reverse isotope dilution with authentic substances. These metabolites accounted for 5% of the urinary radioactivity in man, 15% in the rat and 25% in the dog. A small amount of the primary amine formed by *N*-dealkylation of Ro 3-3528 was found in rat urine. This compound was found to be a good substrate for mitochondrial monoamine oxidase. Since treatment of rats with the monoamine oxidase inhibitor Pargyline had only a slight effect on the quantity of primary amine excreted after Ro 3-3528 had been given, oxidative deamination of this metabolite is catalysed by other enzymes.

The other identified metabolite from rat urine was a phenol derived by hydroxylation of Ro 3-3528 in the 5- or possibly the 4-position. Identification was based on mass-spectral, n.m.r. and u.v. measurements and on changes in the acid-lability of the benzofuran ring. This compound made up 25% of the metabolites in the urine in the rat, 5% in the dog and was not detected in human urine.

In man 90% of administered radioactivity appeared in urine in 48 hr. In the rat the 6-carboxy metabolite was excreted as rapidly in bile as in urine, despite its relatively low molecular weight (278).

Distribution of the drug was studied in the mouse by whole-body radioautography. The duration of β -blocking activity correlated with the decline of tissue drug concentrations as measured fluorimetrically. These concentrations were much higher in female than male rats and in male than female mice, in accord with known sex-determined differences in drug-metabolizing enzymes in these species.

Analogous benzofurans lacking a 6-substituent were metabolized in the rat by 6-hydroxylation. For example, the metabolites of 2-(1-hydroxy-2-isopropylaminoethyl)benzofuran in the urine were the glucuronide of the 6-hydroxy compound (75%) and a mixture of acids obtained by oxidative deamination (25%).

Quinate Metabolism by *Lactobacilli*

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Lactobacillus pastorianus, a typical heterofermentative cider lactobacillus, reduces (–)-quinate and shikimate to dihydroshikimate (Carr, Pollard, Whiting & Williams, 1957). The enzyme system responsible has been found to be adaptive. Adapted washed cells incubated anaerobically with quinate or shikimate, in the absence of a hydrogen donor, formed an equimolecular mixture of the two. In the presence of quinate or shikimate, the rate of CO₂ formation from fructose by adapted cells was increased sevenfold. Of the usual products of fructose metabolism, namely mannitol, lactate, acetate and CO₂, neither mannitol nor lactate was found, only acetate and CO₂, together with dihydroshikimate. In the absence of shikimate washed cells metabolized glucose at only one-twentieth the rate of fructose, but in its presence the rates of CO₂ formation from glucose and fructose were identical. Aerobically, washed cells in the absence of an artificial electron acceptor oxidized lactate