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-ylglycollic 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 (–)-quinic acid 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

slowly, but anaerobically both D(-)- and L(+)-lactate were rapidly oxidized to acetate by the shikimate $\rightarrow$ dihydroshikimate reduction. The reaction proceeded stoichiometrically as follows:

A flavin compound may be involved since addition of riboflavin, FMN or FAD accelerated the reaction. Also, addition of oxalate, an inhibitor of NAD-independent lactate dehydrogenases (Snoswell, 1963), initially strongly inhibited oxidation of L-lactate by the shikimate reductase system. It was not found possible to bring about the reverse process, oxidation of dihydroshikimate to shikimate aerobically, even in the presence of phenazine methosulphate.

The suggestion that quinate $\rightleftharpoons$ shikimate $\rightleftharpoons$ dihydroshikimate might act similarly to malate $\rightleftharpoons$ fumarate $\rightleftharpoons$ succinate as an oxidation-reduction system in plant tissues was made earlier (Hulme, 1951).

Homofermentative *lactobacilli* showed a more complex pattern of quinate and shikimate metabolism. Adaptive enzyme systems resulted in the formation of an acidic product (I) and of catechol. The acid (I) was obtained with m.p. 150° and $[\alpha]_D^{18.5} -18^\circ$ (c 1.0 in water). It was identified by mass and n.m.r. spectra, together with acetyl lactone formation, as all-*cis*-3,4-dihydroxycyclohexane-1-carboxylic acid. Dihydroshikimate also was reduced to acid (I), but no catechol was formed. Both D- and L-lactate were oxidized anaerobically by adapted cells with concomitant reduction of quinate, shikimate or dihydroshikimate to acid (I).

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Metabolites of Sulphadimethoxypyrimidine in the Urine of Various Species

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2,4-Dimethoxy-6-sulphanilamidopyrimidine (sulphadimethoxine) is excreted by man and other primates mainly as an N^1 -glucuronide (Adamson, Bridges & Williams, 1966) in which glucuronic acid is attached to the sulphonamide nitrogen (N^1)

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(Bridges, Kibby & Williams, 1965). This glucuronide is formed in only minor amounts in several lower mammals and not at all in rabbits (Bridges, Kibby, Walker & Williams, 1968). Further, if the positions of the methoxy groups in the pyrimidine ring of the drug are altered or if one is removed from the 2- or 4-position, the ability to form an N^1 -glucuronide *in vivo* in man or the rhesus monkey is considerably diminished or abolished (Bridges, Kibby, Walker & Williams, 1969). If the 2- and 4-methoxyl groups are replaced by methyl groups N^1 -glucuronide formation is abolished, but if the 2-methoxyl group only is replaced by a methyl group, as in 4-methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine), the N^1 -glucuronide formation still occurs in man and the monkey (Bridges, Walker & Williams, 1969). The formation of N^1 -glucuronides in man and monkey seems to be associated with the presence of certain groups (e.g. methoxyl) in the two positions *meta* to the attachment of the sulphanilamido group to the pyrimidine ring. This arrangement is found in the drug sulphadimethoxypyrimidine or 4,6-dimethoxy-2-sulphanilamidopyrimidine.

This drug was administered orally to monkey, rabbit, rat (each 100 mg./kg.) and man (30 mg./kg.). In the rabbit 66% of the dose was excreted in the urine in 24 hr., but no N^1 -glucuronide was excreted. The main metabolites were the unchanged drug and its N^4 -acetyl derivative in roughly equal amounts (25–30%). In two rhesus monkeys 50 and 70% of the dose was excreted in 24 hr. and the main metabolites now were the N^4 -acetyl derivative and the N^1 -glucuronide in roughly equal amounts (20–25% of the dose). In man and the rat the drug was very slowly excreted, man excreting 10% and the rat 6% of the dose in 24 hr. In man the main metabolite was the N^1 -glucuronide, which amounted to 2.9% of the dose in 24 hr., whereas in the rat more than half the 24 hr. excretion was unchanged drug, the N^1 -glucuronide amounting to 0.4% of the dose.

Thus the N^1 -glucuronide is a major metabolite of sulphadimethoxypyrimidine in the urine of the rhesus monkey, but this metabolite is not formed at all in the rabbit.

A comparison of the physical properties of the three drugs forming N^1 -glucuronides in primates, i.e. 2,4-dimethoxy-6-sulphanilamidopyrimidine, 4-methoxy-2-methyl-6-sulphanilamidopyrimidine and 4,6-dimethoxy-2-sulphanilamidopyrimidine, provided no information on the structural specificity of this conjugation.

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