Epoxide-diol pathway in the metabolism of tricyclic drugs

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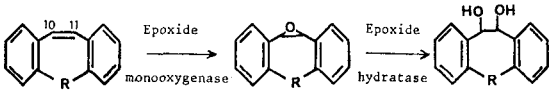
Aromatic and olefinic compounds can be metabolized by mammalian monooxygenases to arene oxides and epoxides respectively (Jerina & Daly, 1974). The labile arene oxides, aromatic compounds in which a formal aromatic double bond has undergone epoxidation, are considered "bioactivated intermediates". As such they are implicated as agents responsible for the toxic, carcinogenic and mutagenic effects attributed to the parent compounds (Jerina & Daly, 1974). The concern that clinically useful drugs containing aromatic and/or olefinic groups may be transformed to bioactivated intermediates led us to examine the metabolism of several tricyclic psychopharmacological drugs.

Previous reports (for a review see: Frigerio, Cavo-Briones & Belvedere, 1976) were concerned with the metabolism of the following drugs: carbamazepine (an anticonvulsant), cyproheptadine (an antihistamine and antiserotoninergic), cyclobenzaprine and protriptyline (antidepressants) (Fig. 1). We have now extended our investigations in a study of the *in vivo* and *in vitro* metabolism of three related drugs, in rat and with rat liver microsomes respectively. The antidepressants opipramol, intriptyline and the anticonvulsant cytenamide, and two model compounds 5H-dibenzo(b, f)-azepine and 5H-dibenzo(a,d)cycloheptene (Fig. 1) were chosen.



5H-dibenzo(b,f)azepine and its derivatives:

R=	>NH	5H-Dibenzo(b,f)azepine
R=	>$NCONH_2$	Carbamazepine
R=	>$NCH_2CH_2CH_2N$⟨⟩NCH_2CH_2OH	Opipramol

5H-dibenzo(a,d)cycloheptene and its derivatives:

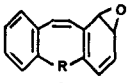
R=	>CH_2	5H-dibenzo(a,d)cycloheptene
R=	>$CHCONH_2$	Cytenamide
R=	>$CHCH_2CH_2CH_2NHCH_3$	Protriptyline
R=	>$C{=}CHCH_2CH_2N(CH_3)_2$	Cyclobenzaprine
R=	>$C{=}CHC{\equiv}CCH_2N(CH_3)_2$	Intriptyline
R=	>$C{=}C$⟨⟩NCH_3	Cyproheptadine

FIG. 1. Major sequence of biotransformation of the tricyclic drugs.

In all the cases studied the major biotransformation was by epoxidation of the 10,11-double bond. The structure of these epoxides was elucidated by gas chromatography-mass spectrometry and, in most cases, confirmed by direct comparison with authentic compounds. Thus, epoxidation is a general biotransformation of these compounds. Another general but much less important step is the conversion of these epoxides to the corresponding diols. These were identified from their mass spectra before and after treatment with *n*-butylboronic acid (Frigerio & others, 1976).

The relative stability of these epoxides *in vivo* and *in vitro* is surprising. Cyproheptadine-10,11-epoxide was found to be a competitive inhibitor of epoxy hydratase (Pachecka, Salmona & others, 1976), an observation which may prove to be valid for the other epoxide metabolites of this series. Evidence is also available that the 10,11-epoxides of carbamazepine, cyclobenzaprine and cyproheptadine are not mutagenic to *Salmonella typhimurium* TA 1537 and TA 1538, whereas the K-region epoxides of some known carcinogens are (Glatt, Oesch & others, 1975). Preliminary studies indicate that these epoxides are not cytotoxic to cell cultures (Frigerio & Morselli, 1975; and unpublished results).

In view of this it is interesting to speculate on the cause of the toxic side effects induced by carbamazepine (Crill, 1973) and cyproheptadine (Wold & Fisher, 1972). Furthermore, the known metabolites of these drugs account in general for only a small percentage of the drug administered. In this context we have observed the presence of other oxygenated compounds among the metabolites of carbamazepine, cyproheptadine, protriptyline and cyclobenzaprine. Chromatographic and mass spectrometric evidence show that these compounds do not arise from modification of the side chain. Furthermore these compounds did not react with bis-trimethylsilylacetamide, expected for phenols. A tentative structure proposed for these new metabolites is that of an arene oxide e.g. (I) but other isomers are possible.



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If the presence of these arene oxides can be established it is tempting to consider whether the formation of these bioactivated intermediates explains those cases in which use of some of these drugs leads to toxic side effects.

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Salicylates, copper complexes, free radicals and arthritis

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I would like to suggest that salicylates and copper complexes with various ligands protect synovial fluid from degradation and leucocytes from premature death by biologically-generated hydroxyl radicals during the treatment of arthritis.

Quick (1974) has pointed out the efficacy of salicylates in the treatment of inflammatory arthritis. McCord (1974) has shown that superoxide radicals reacted with hydrogen peroxide to produce hydroxyl radicals which depolymerized purified hyaluronic acid and bovine synovial fluid. Since phagocytizing polymorphonuclear leucocytes present in the synovial fluid produce superoxide radicals with attendant generation of hydrogen peroxide and hydroxyl radicals, the above reaction was suggested as the *in vivo* mechanism of synovial fluid degradation in inflamed joints. Superoxide dismutase and catalase protected synovial fluid from degradation *in vitro*. In addition, mannitol, an effective hydroxyl radical scavenger, prevented the depolymerization of hyaluronic acid. These same reagents have been shown (Salin & McCord, 1975) to protect phagocytosing leucocytes from premature death and release of hydrolytic enzymes and chemotactic factors which play a role in perpetuating the inflammatory cycle. Fridovich (1975) has suggested that superoxide dismutase, injected into an inflammed area, might minimize the damage caused by superoxide anion secreted by the phagocytes.

Salicylate reacts very readily with hydroxyl radicals (Amplett, Adams & Michael, 1968). In fact, the *ortho*-hydroxy group of salicylate activates the molecule for reaction with hydroxyl radicals when compared with the reaction of hydroxyl radicals with benozate. It is suggested that salicylate acts as a scavenger for hydroxyl radicals generated from superoxide radical resulting from phagocytizing polymorphonuclear leucocytes in synovial fluid. Interestingly enough, cupric salicylate had a greater anti-oedemic effect than sodium or zinc salicylates when administered subcutaneously to rats (Rainsford & Whitehouse, 1976). Furthermore, Sorenson (1976) has demonstrated that cupric ion and copper complexes with a variety of ligands including amino acids and aspirin, administered subcutaneously to rats, were effective anti-inflammatory agents. In this regard, cupric ions and cupric amino acid chelates have been shown to catalyse the dismutation of superoxide anion, a reactant in the generation of hydroxyl radical (Brigelius, Spottl & others, 1974; Brigelius, Hartmann & others, 1975). In part, the mechanism of action of salicylates in the treatment of arthritis may be to protect the synovial fluid, leucocytes and other sensitive elements from attack by hydroxyl radicals. Also, the efficacy of cupric ion and copper complexes as anti-inflammatory agents may, in part, be due to dismutation of superoxide anion.

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