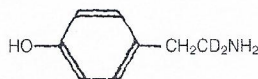
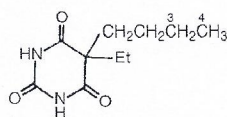


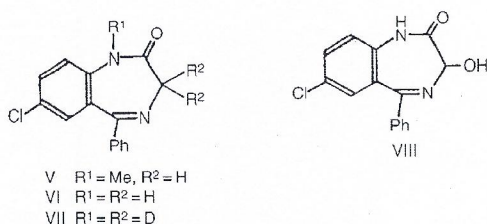
**Fig. 1.** Drugs whose metabolism and action are affected by deuteration. (a) Tyramine (dideuterated form). (b) Butethal. (c) Diazepam (V) is demethylated to form VI, which is then 3-hydroxylated to give oxepam (VIII), which causes the drug's anticonvulsive action. Dideuteration to give form VII reduces 3-hydroxylation and the consequent anticonvulsive action. (d) Antipyrine. (e) Methoxyfluorane and its metabolism by hydroxylation of the dichlorodifluorethyl and methyl groups. (f) Sevoflurane and its metabolites. Figs. 1a–1c, from Foster 1984; Figs. 1d and 1e, from Foster 1985; and Fig. 1f, from Baker et al. 1993.



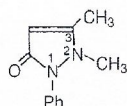
**(a) Tyramine (dideuterated form)**



**(b) Butethal**



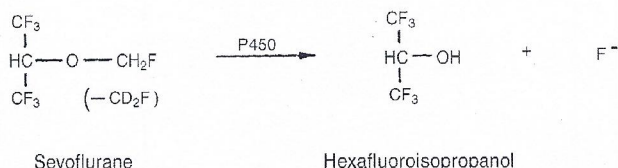
**(c) Diazepam (V) and Oxepam (VIII)**



**(d) Antipyrine**



**(e) Methoxyfluorane and its metabolites**



**(f) Sevoflurane and its metabolites**

acrolein and phosphoramidate mustard might be the actual teratogenic agents.

The metabolic products of many drugs are toxic, and deuteration can lower such toxicity by reducing metabolism. The deuterated forms of a number of halogenated anesthetics have been shown to retain their desirable pharmacological properties but to lose their toxicity. This was shown for the deuterated methoxyfluoranes (Fig. 1e) (McCarty et al. 1979). Deuteration reduced the release of inorganic F from the anesthetic sevoflurane (Fig. 1f) by rat liver microsomes, a process involving the cytochrome P450 system. Inorganic F levels in the blood of treated rats were also lowered (although to a lesser extent than in the in vitro system), and liver damage was reduced (Baker et al. 1993). The toxic effects of chloroform (CHCl<sub>3</sub>) on liver and lungs are thought to be due to its conversion to phosgene (OC(=O)Cl<sub>2</sub>). This conversion by mouse liver microsomes was significantly reduced by deuteration (Nelson 1982).

Van Langenhove (1986) pointed out that the ability of deuterium labeling to change drug metabolism must be kept in mind in using such drugs in pharmacokinetic studies and studies of drug and enzyme metabolism.

In addition to the anesthetic agents just discussed, deuteration reduces toxicity of butylated hydroxytoluene, an antioxidant (Foster 1984), and of diethyl hydroxy adipate, which is a potential food toxin (Loftus et al. 1993). Tamoxifen is widely used for treatment of human breast cancer, but can also cause liver cancers in rats. This is thought to be related to  $\alpha$ -hydroxylation of part of the tamoxifen molecule, converting it to a DNA adduct. The idea was supported by findings that deuterated tamoxifen, which has lower genotoxicity than the hydrogenated form, was also less susceptible to hydroxylation (Jarman et al. 1995).

Studies with the deuterated flame retardant tris(2,3-dibromopropyl)phosphate suggested that the genotoxicity of this substance was due in part to bioactivation by cytochrome P450 (van Beerendonk et al. 1995).

## Deuterated antimicrobial compounds

Certain deuterated antimicrobial compounds are less susceptible to breakdown by their target microorganisms and hence are more effective. These include long-chain fatty acids used against fungi (Abrahamsson et al. 1982) and fluoro-D-phenylalanine, which acts on a number of bacteria (Merck and Co. 1977). The former agents are broken down by  $\beta$ -oxidation, the latter agent by D-amino acid oxidase. Each of these enzymic actions involves breaking a C–H bond, so that it is quite understandable that compounds possessing the more resistant C–D bond are less easily inactivated.

Such results raised the possibility that the deuterated forms of some widely used antibiotics might be less susceptible to microbial attack. The great interest in this arises from the fact that one of the major mechanisms of bacterial resistance to antibiotics, an increasingly important medical problem, involves enzymic changes of the antibiotics by resistant bacteria.

This subject has been very little studied. The first fully deuterated antibiotic, griseofulvin, an antifungal agent, was prepared by growth of *Penicillium janczewskii* (Nona et al. 1968). The deuterated form possessed somewhat more



antifungal activity, against *Microsporium gypseum*, than the protio-form. The authors speculated that this might be due to greater resistance to breakdown by the fungus, but did not explore the subject further.

Deuterated penicillin was prepared some years ago by both chemical (Laskar and Mrtek 1970) and microbiological processes, using *Penicillium chrysogenum* grown on D<sub>2</sub>O with nondeuterated substrates for partially deuterated penicillin, and deuterated substrates for highly deuterated penicillin (Carlstedt et al. 1973). When tested on *Sarcina lutea*, the highly deuterated penicillin was found to have about 80% the activity of the protio-penicillin. Its relative susceptibility to  $\beta$ -lactamase activity was not studied. However, since  $\beta$ -lactamase breaks a C-N bond (Franklin and Snow 1989), there is no reason to think that the deuterated form should be especially resistant to this enzyme.

Unfortunately, detailed consideration of the chemical changes involved in other major cases of bacterial resistance to antibiotics which involve antibiotic destruction suggests that the deuterated forms would not be able to resist such destruction. Chloramphenicol is commonly inactivated by chloramphenicol acetyltransferase, which replaces the H on an OH group by acetyl. Aminoglycosides such as streptomycin and kanamycin are inactivated by phosphorylation and acetylation of certain OH groups and acetylation of NH<sub>2</sub> groups (Franklin and Snow 1989). D atoms on OD and ND<sub>2</sub> groups are readily exchangeable with H of H<sub>2</sub>O (Thomson 1963; Katz 1965) and would not last long in the animal body.

## Use of deuterated insecticides

Deuterated insecticides might have a hitherto unexplored utility. The ability of insects to develop resistance to synthetic insecticides greatly reduces the latter's effectiveness. About half the known examples of such resistance are catalyzed by insect monooxygenases using the cytochrome P450 system. There were so many examples that they were cited as covering 27 pages in a 1985 review (Soderlund and Blomquist 1990). One would expect deuterated insecticides to be more effective than hydrogenated ones, but this subject, which certainly seems worth exploring, has not, to our knowledge, been studied.

## Concluding remarks

Over the years, much work has been carried out, widely and at intervals, on the physiological and pharmacological effects of D<sub>2</sub>O on humans and other animals. Interesting phenomena have been discovered, often without detailed follow-up of some of the questions posed. Action of deuterated drugs, a less complex subject, has been more easily understood, although with few practical therapeutic consequences.

In his review on metabolism of deuterated drugs, Foster (1984) remarked that Blake et al. (1975) in a review 10 years earlier had commented "At the present time there are no drugs on the market that contain deuterium in the molecule" and that the situation had not changed since. Some 15 years after Foster's review, this still seems to be the case. A deuterated drug would, for regulatory purposes, still be considered a new one, and very great expenses are needed to bring any new drug intended for humans to the market. Presumably this would be so, even though past experience has shown how safe D<sub>2</sub>O and deuterated compounds are when used as biochemical tracers. These reviewers hope that continuing research on deuterated drugs will reveal some worth of the effort and expense needed to put them on the market.

Therapeutic treatments of animals with large amounts of D<sub>2</sub>O have yielded intriguing results, but (with the possible exception of potentiating boron neutron capture therapy) are still in the experimental stage as far as regular use on humans is concerned. Some deuterated antimicrobial drugs have a definite but limited use. It seems unlikely that deuterated antibiotics would be more effective than the protonated forms, especially as regards counteracting microbial resistance.

A greater potential might well exist for deuterated insecticides, which could possibly counter one of the most common mechanisms of insect resistance to these compounds. Presumably, regulatory approval of these deuterated forms would be less difficult to obtain than approval for compounds to be used on humans.

In purer scientific terms, no apology is needed for the use of deuterated compounds. Their use in studies of drug metabolism is very well established, as is their contribution to studies of other biochemical pathways. D was one of the very first isotopes to be used in such studies (Shemin 1987), and it is a key element in many biochemical and structural studies (Kushner et al. 1997; LeMaster 1990; Vanatulu et al. 1993). The future need for large quantities of D<sub>2</sub>O in nuclear reactors may be a matter of debate. The continuing need for this compound, and for deuterated compounds in general, in physiological, pharmacological, and biochemical studies, cannot be questioned.

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