REVIEW / SYNTHÈSE

Pharmacological uses and perspectives of heavy water and deuterated compounds

D.J. Kushner, Alison Baker, and T.G. Dunstall

Abstract: Since the discovery of D_2O (heavy water) and its use as a moderator in nuclear reactors, its biological effects have been extensively, although seldom deeply, studied. This article reviews these effects on whole animals, animal cells, and microorganisms. Both "solvent isotope effects," those due to the special properties of D_2O as a solvent, and "deuterium isotope effects" (DIE), which result when D replaces H in many biological molecules, are considered. The low toxicity of D_2O toward mammals is reflected in its widespread use for measuring water spaces in humans and other animals. Higher concentrations (usually >20% of body weight) can be toxic to animals and animal cells. Effects on the nervous system and the liver and on formation of different blood cells have been noted. At the cellular level, D_2O may affect mitosis and membrane function. Protozoa are able to withstand up to 70% D_2O. Algae and bacteria can adapt to grow in 100% D_2O and can serve as sources of a large number of deuterated molecules. D_2O increases heat stability of macromolecules but may decrease cellular heat stability, possibly as a result of inhibition of chaperonin formation. High D_2O concentrations can reduce salt- and ethanol-induced hypertension in rats and protect mice from gamma irradation. Such concentrations are also used in boron neutron capture therapy to increase neutron penetration to boron compounds bound to malignant cells. D_2O is more toxic to malignant than normal animal cells, but at concentrations too high for regular therapeutic use. D_2O and deuterated drugs are widely used in studies of metabolism of drugs and toxic substances in humans and other animals. The deuterated forms of drugs often have different actions than the protonated forms. Some deuterated drugs show different transport processes. Most are more resistant to metabolic changes, especially those changes mediated by cytochrome P450 systems. Deuteration may also change the pathway of drug metabolism (metabolic switching). Changed metabolism may lead to increased duration of action and lower toxicity. It may also lead to lower activity, if the drug is normally changed to the active form in vivo. Deuteration can also lower the genotoxicity of the anticancer drug tamoxifen and other compounds. Deuteration increases effectiveness of long-chain fatty acids and fluoro-D-phenylalanine by preventing their breakdown by target microorganisms. A few deuterated antibiotics have been prepared, and their antimicrobial activity was found to be little changed. Their action on resistant bacteria has not been studied, but there is no reason to believe that they would be more effective against such bacteria. Insect resistance to insecticides is very often due to insecticide destruction through the cytochrome P450 system. Deuterated insecticides might well be more effective against resistant insects, but this potentially valuable possibility has not yet been studied.

Key words: deuterium, heavy water, D_2O, deuterium isotope effects.

Résumé : Depuis sa découverte et son utilisation comme modérateur dans les réacteurs nucléaires, les effets biologiques du D_2O (eau lourde) ont largement été examinés, quoique souvent de manière superficielle. Le présent article présente une synthèse de ces effets sur les animaux, les cellules animales et les micro-organismes. Il considère les « effets isotopiques du solvant », soit ceux découlant des propriétés particulières du D_2O en tant que solvant, et les « effets isotopiques du deutérium » (EID), qui sont observés dans de nombreuses molécules lorsque H est remplacé par D. La faible toxicité du D_2O envers les mammifères se reflète dans son usage répandu pour quantifier les compartiments aqueux chez les humains et les animaux. Utilisé en fortes concentrations (généralement >20% de poids corporel), le D_2O peut être toxique pour les animaux et les cellules animales; des effets sur le système nerveux, le foie et la formation de différentes cellules sanguines ont été observés. Au niveau cellulaire, le D_2O peut avoir des effets sur la mitose et la fonction membranaire. Les protozoaires peuvent tolérer jusqu'à 70% de D_2O. Les algues et les bactéries

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peuvent croître dans 100% de D_2O et peuvent être utilisées comme sources de nombreuses molécules deutériées. Le D_2O augmente la stabilité thermique des macromolécules, mais peut diminuer celle des cellules, probablement à cause de l'inhibition de la formation de chaperonines. De fortes concentrations de D_2O peuvent réduire l'hypertension induite par l'éthanol ou le sel chez les rats; elles peuvent aussi protéger les souris contre l'irradiation gamma. De telles concentrations peuvent être utilisées dans la thérapie de capture de neutrons par le bore (« boron neutron capture therapy ») pour augmenter la pénétration des neutrons dans les composés de bore liés aux cellules malignes. Le D_2O est plus toxique pour les cellules animales malignes que pour les cellules normales, mais seulement en concentrations trop élevées pour un usage thérapeutique normal. Le D_2O et les médicaments deutériés sont largement utilisés dans les études du métabolisme des médicaments et des substances toxiques chez les humains et les animaux. Les formes deutériées des médicaments ont souvent des actions différentes de celles des formes protonées. De même, certains médicaments deutériés ont des processus de transport qui diffèrent. La plupart de ces médicaments sont résistants aux changements métaboliques, en particulier ceux véhiculés par les systèmes des cytochromes P450. La deutérisation pourrait agir sur la voie du métabolisme d'un médicament (« aiguillage métabolique »), ce qui pourrait augmenter la durée d'action, diminuer la toxicité et aussi réduire l'activité si le médicament était modifié normalement en la forme active in vivo. La deutérisation peut aussi diminuer la génotoxicité du médicament anticancéreux, tamoxifène, ainsi que celle d'autres composés. La deutérisation augmente l'efficacité des acides gras à longue chaîne et de la fluoro-D-phénylalanine en prévenant leur dégradation par des micro-organismes cibles. On a effectué la deutérisation de quelques antibiotiques pour constater que ce processus a eu peu d'effet sur leur activité microbienne. Leur action sur les bactéries résistantes n'a pas été examinée, mais il n'y a pas lieu de croire qu'ils seraient plus efficaces contre de telles bactéries. La résistance des insectes aux insecticides est très souvent attribuable à la destruction de ces derniers par le système des cytochromes P450. Il se pourrait que les insecticides deutériés soient plus efficaces contre les insectes résistants, mais cette intéressante éventualité n'a pas encore été examinée.

Mots clés : deutérium, eau lourde, D_2O, effets isotopiques du deutérium.

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Introduction: historical aspects and properties of D_2O

Deuterium (D) was discovered as a natural isotope in H_2O, which contains 0.015% D_2O (Urey et al. 1932). Its use as a moderator in nuclear reactors provided political and industrial impetus for its large-scale manufacture. This led to production of about 30 000 tonnes of "pure" (actually about 99.8%) D_2O by 1991 (Benedict et al. 1981; Rae 1991; Miller and van Alstyne 1994). All processes of D_2O production require much energy, so that its cost has remained high, still more than $240US per kilogram (Rae 1991).

Even so, enough D_2O has been available for its physical, chemical, and biological properties to be extensively studied. Deuterium (^2H), with twice the mass of a hydrogen atom, is one of the very few isotopes to be given a separate name than the main form of the element. The masses of other stable isotopes (^{13}C, ^{15}N, and ^{18}O), which have been widely used in biological studies, differ little from the predominant forms of each element (^{12}C, ^{14}N, and ^{16}O).

Some of the physical properties of D_2O are listed in Table 1. Briefly, it is denser and more viscous than H_2O and has higher melting and boiling points. Differences in other physical properties are less marked. Deuterium bonds in D_2O are stronger than the analogous hydrogen bonds in H_2O (Katz 1965; Eisenberg and Kauzmann 1969).

Solvent and deuterium isotope effects

Living systems exposed to D_2O experience at least two sets of effects. One is a "solvent isotope effect," because of the properties of D_2O itself, and especially its effects on the structure of water and macromolecules. The second is the "deuterium isotope effect" (DIE), resulting from the ability of D_2O to replace H with D in biological molecules. The C–D bond is about 10 times as strong as the C–H bond and more resistant to chemical or enzymic cleavage. Compounds with C–D bonds tend to remain stable in H_2O indefinitely, and such compounds have been very widely used for isotopic studies. O–D, N–D, and S–D bonds are also stronger than the corresponding protonated forms, but the D in such bonds quickly exchanges with H in H_2O (Katz 1965; Thomas 1971).

Deuterium isotope effects are usually considered only in terms of D linkages to C atoms. Deuteration of O, N, and S in biological molecules must occur rapidly when cells are exposed to D_2O, but the reversibility of these processes by exchange with H^+ makes it very difficult to assess the biological effects of such deuteration.

The ratio of the rates of cleavage of a C-protonated and -deuterated compound, V^H_{max}/V^D_{max}, expresses the "primary" deuterium isotope effect, usually called simply the deuterium isotope effect (DIE) (Northrop 1982; Foster 1985). Tenfold differences in rates are common. "Secondary" deuterium isotope effects occur when attachment of D to another atom affects the rate of C–H cleavage; such effects are usually small. The existence of a DIE in comparing protonated and deuterated compounds has been widely used to show whether metabolic reactions involve cleavage of C–H bonds. This technique was used by Darbyshire et al. (1994) to study mechanisms of testosterone metabolism, by Deraniyagala et al. (1995) to study the mechanisms of chemical hydrolysis of penicillanic acid, and there are many other examples. H–D exchange was used to study antigen–antibody reactions (Paterson et al. 1990) and

Table 1. Some physical properties of heavy and light water (from Katz 1965).

Property	D_2O	H_2O
Melting point (°C)	3.82	0
Boiling point (°C)	101.72	100.0
Density (20°C, g/mL)	1.1056	0.9982
Temp. of maximum density (°C)	11.6	4.0
Viscosity (20°C, centipoise)	1.25	1.005
Surface tension (25°C, dyn·cm)	71.93	71.97
Heat of fusion (cal/mol)	1515	1436
Heat of vaporization (cal/mol)	10 864	10 515

Note: Surface tensions and dielectric constants of H_2O and D_2O are essentially identical. 1 poise = 0.1 Pa·s; 1 dyn = 10 μN; 1 cal = 4.1858 J.

mechanisms of H_2 formation by methanogenic bacteria (Klein et al. 1995). References to the use of D labels in many spectroscopic studies (reviewed in Kushner et al. 1997; LeMaster 1990) illustrate the important contributions this isotope makes to current biological research but are beyond the scope of this review.

It is not always possible to distinguish between solvent isotope and deuterium isotope effects. If growth and cell division, or even biosynthesis take place in the presence of D_2O, some deuterated molecules will be formed. Many experiments with animals, plants, or microorganisms have taken place over periods of days, weeks, or longer, so that substantial amounts of such molecules were made. In contrast, short-term effects on isolated cells or enzymes are likely due to solvent isotope effects alone.

Effects of D_2O on humans and other animals

Shortly after the availability of pure D_2O, its effects on a number of biological systems were extensively studied (Katz 1960; Thomson 1963). Animals were injected with or forced to drink large volumes of D_2O, resulting in high levels of D_2O in their blood and body fluids and presumably in deuteration of many of their molecules. Usually such experiments did not indicate the mechanisms of action involved, and the results will only be described briefly. Some attention will be given to more recent examples that suggest that D_2O may have important potential uses in human therapy.

Mice, rats, and dogs with about 25% D content remained apparently healthy for long periods and produced sperm and eggs, but were sterile. Higher levels of deuteration soon led to acute neurological symptoms, liver hyperplasia, anemia, and other symptoms, and eventually death. Physiological damage, and associated enzymatic changes, seemed to be reversible if the animals were returned to a normal water diet, even though some D remained in certain tissues, especially the brain, for some time. High ambient D_2O concentrations (ca. 90%) rapidly killed fish, tadpoles, flatworms, and drosophila.

More recent studies showed that D_2O ingestion affects formation and properties of mammalian blood cells. It impairs hematopoiesis, lowering formation of platelets, neutrophils, and especially lymphocytes in the mouse (Adams and Adams 1988).

Although high D_2O concentrations are certainly toxic to animals, small amounts are not. It is widely used to measure water space in animals, including humans of all ages (Coward 1979), and can be used as a tracer to measure compliance in drug trials (Rodewald et al. 1989). Commonly, 0.1 mL per killogram body water is swallowed, that is 5–7 mL for an adult human. This increases the D_2O content in the blood from 150 to about 300 ppm, which subsequently decreases to the normal level with a half-life of a few days. No adverse effects have been reported from many such tests (Coward 1979). To reach a level of 10% in body water, which might or might not be toxic, a 70-kg man (with about 50 L body water) would have to drink rapidly 5 L of pure D_2O. This seems unlikely to occur either by intent or by accident. D_2O concentrations as high as 23% in human fluids were found not to be toxic over short time periods (Wallace et al. 1995).

Prospective clinical effects of D_2O

Some studies suggest that high D_2O concentrations might have valuable pharmacological and even clinical effects. Vasdev et al. (1993, 1994) showed that feeding rats fructose or ethanol increased systolic blood pressure, platelet cytosolic free calcium, and aortic calcium uptake. Fructose-fed rats had higher levels of plasma glucose, insulin, and triglycerides. Those fed ethanol showed smooth muscle hyperplasia and thickening of the walls of small arteries and arterioles in the kidney. Inclusion of 10% D_2O in the drinking water of ethanol-fed rats, and 5% D_2O in that of fructose-fed rats, lowered blood pressure and cytosolic free calcium in both groups of animals, counteracted the renal vascular changes in the ethanol-fed rats, and lowered triglycerides in those fed fructose. Possible cellular mechanisms for some of these effects of D_2O are discussed below. A patent has been obtained for the use of heavy water in treating human subjects (Liepins 1993), although it is not clear to what extent this has been employed in actual clinical practice.

Laissue et al. (1987) found that mice maintained on a diet of 29% D_2O were less susceptible to normally lethal doses of whole body gamma irradiation.

Although hemopoietic and lymphoid tissues were equally damaged in deuterated and undeuterated mice, bone marrow and other cells in the former showed accelerated regeneration, suggesting that deuteration protected pluripotent stem cells during irradiation. In contrast, suspension in D_2O increased postradiation damage in V79 Chinese hamster cells (Utsumi and Elkind 1991); possible reasons for this will be discussed below.

The antimitotic action of D_2O (see below) has stimulated its use as an antitumor agents. Effective D_2O concentrations were usually too toxic to animals for rational chemotherapy. Combining D_2O treatment with cytostatic drugs such as methotrexate caused more reduction of tumor growth than either agent alone, although definitive cures did not result (Laissue et al. 1982). A more recent study (Bauer et al. 1995) showed that D_2O was much more effective in killing malignant melanoma and carcinoma cells (colon carcinoma, glioblastoma, and small lung cell cancer cells) than PHA-stimulated lymphocytes and normal glial cells. For example, 90% D_2O killed 70% of the former but only 5% of the latter

group. A differential effect on cell growth also occurred, and 9 days of treatment with 90% D_2O reduced the surviving fraction of malignant cells to about 0.1%. Again, the effective D_2O concentrations are much too high to use in human therapy.

High D_2O concentrations may be valuable in boron neutron capture therapy (BNCT), which is used to treat tumors, especially brain tumors, by neutron irradiation of boron-containing compounds bound to malignant cells. Subsequent emission of α-particles and other radioactive rays selectively kills these cells. The degree of neutron penetration into tissue, which limits the effectiveness of this method, can be greatly increased if the patient's brain is "loaded" with 40% heavy water (Hatanaka 1989, 1991; Wallace et al. 1995). This concentration, which is almost certainly toxic, is maintained only during exposure to a neutron beam, and may be quickly washed out by perfusion with saline in H_2O. Some success was reported in treating these desperate cases.

Effects of D_2O on proteins, cells, and tissues

As a solvent, D_2O increases stability of proteins and other molecules, likely through increasing the formation of hydrophobic bonds (Katz 1965; Kresheck et al. 1965).

D_2O can maintain the stability of vaccines, including the polio vaccine, for long periods without refrigeration (Aldhous 1995). Lower antifreeze activity of glycoproteins of polar fishes in D_2O than in H_2O was considered to be due to a stronger solvent ordering effect of the peptide backbone of these molecules by D_2O than by H_2O (Ahmed et al. 1980). The effect of D_2O on hydrophobic bond formation was thought to cause stabilization of heliozoan microtubule formation (Marsland et al. 1971), and it has been used as an active polymerizer of tubulin in a number of systems (Itoh and Sato 1984; Sollott et al. 1995). D_2O was also shown to cause a decrease in the high salt requirement of halophilic archaebacteria, organisms in which salt plays a major role in stabilizing hydrophobic bonds (Kushner 1998).

Early work (Thomson 1963) showed that D_2O inhibited nerve and muscle actions in isolated systems. Cells in tissue culture could still grow, although more slowly, in 99.8% D_2O. Differential effects on normal and malignant animal cells were discussed above.

D_2O inhibits mitosis in many plant and animal cells. This effect seems due partly to its effect on tubulin polymerization (see above) and also, or especially, on its action on microtubule organizing centres and other structures governing formation of the mitotic spindle (Lamprecht et al. 1991).

Other effects of D_2O on cell structure have been noted. In addition to affecting the formation of different blood cells, including platelets, as mentioned above (Adams and Adams 1988), D_2O also affects platelets in vitro, inhibiting their spreading, retraction, and aggregation by ADP and collagen, and stimulating their adrenaline-induced aggregation (Reuter et al. 1985). These effects on platelet movement were discussed in terms of membrane receptors and energy metabolism. However, the effects of D_2O on microfilament systems, which may be responsible for changes in shape of human neutrophil granulocytes (Zimmermann et al. 1988), might also be involved in the effects on platelets.

Vasilescu and Katona (1986) found that D_2O inhibited bioelectrogenesis and contractility in nerve and muscle preparations and uncoupled electrical and mechanical functions in the isolated frog heart. It lowered the ATP/ADP ratio in all these tissues and also played an antagonistic role to anesthetics in sciatic nerve trunks.

Among other mechanisms considered (differences in hydration of macromolecules and stabilization of large biopolymers) Vasilescu and Katona (1986) made the interesting suggestion that D functioned less well than H in energy production. This would mean that a deuteron motive force would be less effective than a proton motive force, a possibility that has not, to our knowledge, been tested directly.

There are, indeed, few studies of deuteron–proton competition in membrane and enzyme systems. Elsing et al. (1995) found that D_2O only slightly inhibited Na^+–H^+ antiport activity in human leucocytes, and its effect on bile formation was more closely related to interference with Cl^-–HCO_3^- exchange in liver cells.

D–H competition may have important effects on Ca^{2+} channels. Vasdev et al. (1993, 1994) suggested that the antihypertensive effects of D_2O may be related to its ability to reduce L-type calcium channel conductance in myocytes and calcium uptake in rat aortic rings treated with phenylephrine and KCl. Proud'hon et al. (1987) showed that deuterium ions could compete with protons for a single site in the L-type calcium channel of guinea pig ventricular myocytes. Binding and unbinding of protons to this site seem essential for Ca^{2+} movement. Interference by D^+, whose unbinding to this site is about 2.5 times slower than that of H^+, could substantially reduce the flow of current through the L-type Ca^{2+} channels.

D_2O has a number of other effects on membrane function, including membrane depolarization and activation of Ca^{2+} channels in algae, inhibition of Na^+–K^+ ATPase in animal membranes and interference with Cl^-–HCO_3^- exchange in liver cells (Andjus and Vucelic 1990; Andjus et al. 1994; Elsing et al. 1995). These effects do not seem to have been studied in depth.

D_2O is much less toxic to microorganisms than to multicellular creatures. After a period of adaptation, a number of bacteria and algae can grow in pure D_2O, although usually more slowly than in H_2O (Thomson 1963; Katz 1960, 1965; Crespi 1982; Unno et al. 1989; Vanatulu et al. 1993; Haon et al. 1993; Kushner et al. 1997). More complex cells, such as protozoa, can grow in 70% or higher D_2O (Thomson 1963). Microorganisms growing in D_2O produce a large number of deuterated compounds. Algal cells (*Chlorella* spp.) growing in different D_2O concentrations usually, although not always, show a preference for H over D incorporation into cellular biomass (Unno et al. 1987).

Hydrolysates of deuterated algae can also serve as feedstock for the growth of heterotrophic microorganisms, some of which might produce interesting pharmacological compounds (Crespi 1989).

Although D_2O increases heat stability of macromolecules, it does not always increase that of cells. Earlier work (reviewed by Unno et al. 1989) showed that D_2O sometimes increased and sometimes decreased heat sensitivity of mammalian cells. Unno et al. (1989) found that H_2O-grown

Chlorella ellipsoidea were less heat sensitive in D_2O than in H_2O, and that the sensitivity declined with increasing D_2O concentrations. However, D_2O-grown *Chlorella ellipsoidea* were more sensitive than H_2O-grown ones in H_2O.

The increased heat sensitivity of deuterated *Chlorella ellipsoidea* seems due to a decreased ability to synthesize heat shock proteins, and (or) to increased susceptibility of deuterated proteins to heat denaturation (Unno and Okada 1994). The ribulose 1,5-bisphosphate carboxylase (Rubisco) from deuterated *Chlorella ellipsoidea* was less active than the enzyme from H_2O-grown cells, but that activity could be restored by addition of chaperonin proteins, the GroE proteins of *Escherichia coli*. This suggested that deuteration had made functional assembly of Rubisco difficult (Yokogaki et al. 1995). Earlier, D_2O had been shown to prevent formation of heat shock proteins in cultured chicken embryo cells (Edington et al. 1989).

One little-studied effect of D_2O is its ability to stabilize (in relative terms) highly reactive and toxic forms of oxygen. Singlet oxygen exists about 10 times as long (120 μs compared with 12 μs) in 90% D_2O as in H_2O (Rodgers and Snowden 1982). The increased existence of this form of oxygen was thought to be involved in the increased photosensitized destruction in D_2O of human bladder carcinoma cells after treatment with the photosensitizing agent chlorine e_6 (Bachor et al. 1991). It could also be related to enhanced killing of V70 Chinese hamster cells after ionizing radiation, when these are suspended in 90% D_2O (Utsumi and Elkind 1991).

Properties and uses of deuterated drugs

As already stated, the C–D bond is more stable than the C–H bond, and such D in organic compounds is not readily exchangeable in H_2O. Deuterated organic compounds can be detected with great sensitivity by mass spectrometry and other methods. Because of these considerations, and the very low toxicity of deuterated compounds (especially compared with radioactive ones) such drugs, as well as D_2O, are very widely used in studies of metabolism and movement of drugs and toxic substances in humans and other animals.

Some specific examples of special interest include the antitumour agents RSU 1069 and Ro 03-8799, electron-affinic radiosensitizers that bind DNA. Deuterated (and tritiated) forms of these compounds were synthesized, to help in the studies of their metabolic activities (Webb and Threadgill 1990).

Deuterated (and tritiated) analogs of the alkaloid camptothecin, another antitumor agent that inhibits topoisomerase I, a key enzyme in DNA synthesis, have also been prepared (Hinz et al. 1996).

Deuterated analogs of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which is oxidized to more toxic species, were prepared to study the pathway that leads to these changes (Mabic and Castagnoli 1996). Dideuterated analogs of nordiazepam and diazepam were prepared to study isotope effects in oxidative reactions catalyzed by drug-metabolizing enzyme systems (Yang et al. 1996). Deuterated amines (Tsuzuki et al. 1996), nonsteroidal antiinflammatory 2-arylpropionic acids (Castell et al. 1994), the mucolytic drug domiodol (Ferraboschi et al. 1994), and artemisinins, anti-malarial drugs (Avery et al. 1996) were synthesized to aid in the study of the metabolism and movement of these drugs. Deuterated penicillamine was prepared to aid in the nuclear magnetic resonance spectroscopy of peptide hormones and neutrotransmitters (Mosberg et al. 1987).

Other examples are given in the papers of Nelson (1982), Vandenheuvel (1982), Loftus et al. (1993), Scobie et al. (1994), Silvestro et al. (1994), Mazier and Jones (1997), and Munro et al. (1997). This list of references is by no means exhaustive, and only suggests the range of substances studied. A substantial portion of the articles in each current issue of the *Journal of Labelled Compounds and Radiopharmaceuticals* deals with methods of preparing deuterated drugs and other active organic compounds.

Drugs of special interest are those whose deuterated forms have different pharmacological effects than the fully hydrogenated forms. This subject was reviewed by Foster (1984, 1985), first briefly, then in much depth, with detailed treatment of the mechanisms involved. Sometimes changed properties are due to different transport or penetration of the drug itself. Wenzel (1989) showed that deuterated amphetamines (also labelled with ^{131}I) were taken up more readily into mouse and rat brains than the hydrogenated forms of the same compounds. More often, changes in pharmacological action seem due to changes in drug metabolism. Of special interest are reactions catalyzed by the hepatic cytochrome P450 system, the monooxygenases that act on halogenated and nonhalogenated drugs (Archakov and Bachmanova 1990). One of the key steps in all such reactions is the breaking of a C–H bond, and compounds that have C–D structures at the site of enzymatic attack are more resistant to P450 change.

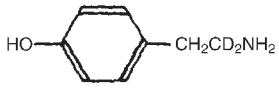
Resistance to P450-induced changes may lead to an increase in duration of pharmacological action. A very early example of this was a doubling of the action time of the adrenergic drug *p*-tyramine (Fig. 1*a*), by deuteration. A similar effect was found when the barbiturate butethal (Fig. 1*b*) was deuterated at the position normally subjected to 3-hydroxylation (Foster 1984, 1985).

Sometimes, however, oxidation leads not to the destruction of a drug but to the formation of the active compound. In such a case, deuteration can reduce the drug's activity. An example is diazepam (Fig. 1*c*), which requires 3-hydroxylation to oxepam for its anticonvulsive action. Diazepam, which is dideuterated at position 3, has lower anticonvulsive action, which may be due to the lower degree of 3-hydroxylation (Foster 1984).

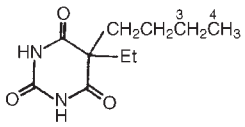
Deuteration can also cause "metabolic switching," whereby the paths of metabolism of a drug are quantitatively changed. The major route of antipyrine (Fig. 1*d*) metabolism is by oxidation of the C3 methyl group, with a small amount of de-N-methylation. However, if antipyrine is deuterated on the C3 methyl group, this group is little oxidized (in rats), and the main pathway is by demethylation (Foster 1984, 1985).

Spielmann and Nau (1986) studied the metabolic switching of cyclophosphamide following deuteration to help define the metabolic pathway needed for teratogenic activation of this drug. Their results suggested that the metabolites

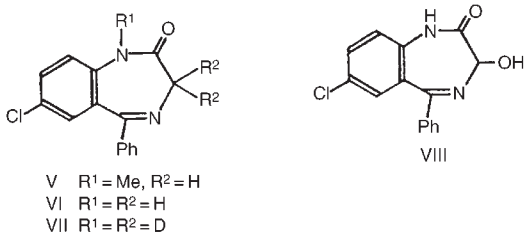
Fig. 1. Drugs whose metabolism and action are affected by deuteration. (*a*) Tyramine (dideuterated form). (*b*) Butethal. (*c*) Diazepam (V) is demethylated to form VI, which is then 3-hydroxylated to give oxepam (VIII), which causes the drug's anticonvulsive action. Dideuteration to give form VII reduces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



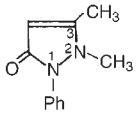
(a) Tyramine (dideuterated form)



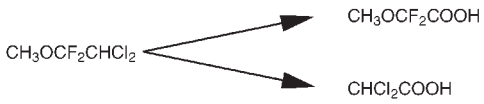
(b) Butethal



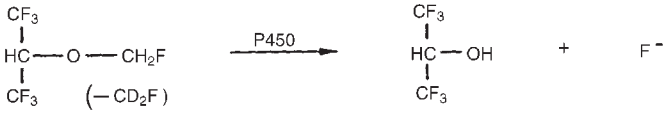
(c) Diazepam (V) and Oxepam (VIII)



(d) Antipyrine



(e) Methoxyfluorane and its metabolites



(f) Sevoflurane and its metabolites

acrolein and phosphoramide 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 methoxyfluranes (Fig. 1*e*) (McCarty et al. 1979). Deuteration reduced the release of inorganic F from the anesthetic sevoflurane (Fig. 1*f*) 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 ($CHCl_3$) on liver and lungs are thought to be due to its conversion to phosgene ($OCCl_2$). 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 pharmokinetic 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 α-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 β-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 *Microsporum 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_2O 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 protiopenicillin. Its relative susceptibility to β-lactamase activity was not studied. However, since β-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_2 groups (Franklin and Snow 1989). D atoms on OD and ND_2 groups are readily exchangeable with H of H_2O (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 monooxgenases 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_2O 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_2O and deuterated compounds are when used as biochemical tracers. These reviewers hope that continuing research on deuterated drugs will reveal some worthy of the effort and expense needed to put them on the market.

Therapeutic treatments of animals with large amounts of D_2O 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_2O 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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