THE SUBCELLULAR DISTRIBUTION OF DEFLUORINATION ACTIVITY IN MARSUPIAL AND RODENT SPECIES.

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ABSTRACT

Defluorination activity in liver subcellular fractions was investigated in species of rodent, marsupials and monotremes. The defluorination activity in the mouse and rat was compared with that of two Australian marsupials, the brushtail possum and tammar wallaby to determine the species-related differences in fluoroacetate sensitivity. The results indicated that there are clear species differences in the subcellular distribution of defluorination activity amongst the four species. While the cytosolic fraction exhibited the major proportion of defluorination activity in all species, the marsupials possessed a certain amount of defluorination ability in their mitochondrial fractions. The nuclear fractions generally contained a consistent amount of defluorination activity across the four species, indicating the need for further investigation. The single platypus and echidna samples also exhibited a similar distribution of defluorination activity in the liver subcellular fractions. These results clearly localise the fluoroacetate defluorination activity in liver cytosol and provide further insight into the fluoroacetate detoxication ability of Australian marsupials and monotremes.

The enzyme kinetic study revealed that the Australian marsupials living outside the range of fluoroacetate-containing plants appear to have a lower ability to detoxify fluoroacetate than rodents have. This suggests that marsupials may be more disadvantaged in their metabolic detoxication of some xenobiotics than previously thought.

Key words: fluoroacetate; subcellular fraction; defluorination activity; Australian marsupial.

INTRODUCTION

Fluoroacetate (compound 1080) is a common mammalian pest control agent and is highly toxic to a wide range of animals, including particular non-target species such as unadapted eutherian mammals (Twigg and King 1991, 2000; Seawright and Eason 1994). It has been used in Australia and New Zealand to reduce the impact of vertebrate pests on agricultural production, and on native plants and animals. Fluoroacetate is also a naturally occurring toxin in some plant genera in Africa (*Dichapetalum*), South America (*Palicourea*) and Australia (*Gastrolobium*, *Nemcia* and *Acacia*) (Crisp and Weston 1994; Twigg *et al.* 1996).

Fluoroacetate is highly toxic to mammals due to its sequential metabolic conversion to fluoroacetyl coenzyme A and fluorocitrate, which inhibits aconitate hydratase and blocks the Krebs cycle at the citrate stage. Fluoroacetate inhibits citrate transport through the mitochondrial membrane (Mead *et al.* 1979; Toninello *et al.* 1983; Twigg *et al.* 1986). Fluoroacetate also forms a thiol-ester bond with the sulfhydryl groups of two glutathione-dependent enzymes in the mitochondrial membrane (Kun *et al.* 1977; Kirsten *et al.* 1978). Fluoroacetate toxicity results in citrate accumulation in most tissues, including an increase in plasma citrate concentration, and ultimately energy deprivation, destruction of cellular permeability barriers and loss of function, leading to organ disorders and eventual death (Eason *et al.* 1994; Mead *et al.* 1979; Twigg *et al.* 1986). Since the liver accumulates far less citrate than other highly metabolically active tissues (Buffa and Peters 1949; Potter and Busch 1950), the liver may have a detoxication function that protects it against fluoroacetate intoxication.

Detoxication of fluoroacetate by defluorination is known to occur in plants, bacteria and a variety of animals (Smith *et al.* 1977; King *et al.* 1978; Mead *et al.* 1979; Twigg *et al.* 1986). *In vivo* defluorination of fluoroacetate or its metabolites has been demonstrated in laboratory rats (Smith *et al.* 1977; Kostyniak *et al.* 1978), in red kangaroos (*Macropus rufus*) and grey kangaroos (*Macropus fuliginosus*) (Oliver *et al.* 1977) and in Australian possums (*Trichosurus vulpecula*) and bush rats (*Rattus fuscipes*) (King *et al.* 1978). However, these studies used liver acetone powder to measure defluorination activity and did not give an indication of the actual subcellular distribution of enzymatic defluorination. Kostyniak and co-workers (1978) had previously used the *in vitro* fluoride release assay to determine the capability of rat liver homogenates to defluorinate fluoroacetate, and for comparing the defluorination reaction with known dehalogenation systems in rat liver. In further studies, they also compared the organ distribution of the fluoroacetate-defluorinating enzyme in the liver, kidney, lungs, heart and testes of the *CFW Swiss* mouse, and found that mouse liver demonstrated the highest defluorination activity among the organs examined (Soiefer and Kostyniak 1983).

Since the 1970s, a series of reports have indicated that some Australian mammals (and other animals) living within the range of fluoroacetate-containing plants are unusually tolerant to fluoroacetate, while corresponding species living outside the range of these plants are far more sensitive to the toxin. There can be as much as a hundred-fold difference in sensitivity to fluoroacetate toxicity between species and even within species, yet there is inadequate information to explain the metabolic process underlying this observation (Twigg and King 1991). To understand the mechanism of fluoroacetate detoxication by defluorination, it is important to investigate the distribution of defluorinase activity in subcellular fractions of the liver, the main organ playing a role in defluorination. To date, however, the subcellular distribution of

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fluoroacetate-specific defluorinase (FSD) has only been examined in rat (Kostyniak *et al.* 1978). There has not been any previous investigation of this profile in Australian marsupials.

In mammals, it is believed that the detoxication of fluoroacetate by defluorination occurs mainly in the liver and is catalysed by FSD (Soiefer and Kostyniak 1983; 1984). Defluorination in animals is achieved by a glutathione-dependent enzymatic mechanism that produces free fluoride ion and S-carboxymethylcysteine (Kostyniak *et al.* 1978). The metabolite S-carboxymethylcysteine has been detected in the urine sample of fluoroacetate-treated possums, which suggests that glutathione S-transferase (GST) is the enzyme responsible for fluoroacetate metabolism (Mead *et al.* 1979). Depletion of liver glutathione (GSH) enhances the toxicity of fluoroacetate in animals, also supporting the concept of a GSH-dependent enzymatic detoxication mechanism (Kostyniak 1979). In *CFW Swiss* mice the liver has been shown to be the major detoxifying organ, as it has the most defluorination activity, as well as high GSH and GST levels (Soiefer and Kostyniak 1983). However, the distribution of defluorination activity between subcellular fractions is yet to be investigated in Australian eutherian mammal and marsupial species. Hepatic metabolism of xenobiotics can be affected by gender (Meyer *et al.* 1993; Bonate 1991), as sex differences in mixed function oxidases (MFO) and GST have been reported.

To further investigate the subcellular localisation of FSD in mammal species, especially Australian marsupials, the present study was undertaken in rodents and a number of native Australian mammals which live outside the range of fluoroacetate-containing vegetation. The species and sex related differences in FSD activity were examined to determine some of the factors leading to the species and sex-related differences in fluoroacetate sensitivity, and to provide a scientific basis for understanding the process by which some animals have evolved a tolerance to fluoroacetate.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid (TCA), dithiothreitol (DTT), potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate trihydrate, ethylenediaminetetraacetic acid (EDTA), reduced glutathione, sodium citrate, pentobarbitone sodium (Nembutal), potassium chloride (KCl), sodium fluoride and sodium fluoroacetate were obtained from Sigma Chemical Co. (St. Louis, MO, USA.). Heparin was obtained from CSL Biosciences (Parkville, Vic, Australia).

Experimental animals

Liver samples were obtained from representative species of Rodentia: rats (*Wistar*) and mice (*Balb/c*); Marsupialia: brushtail possum (*Trichosurus vulpecula*) and tammar wallaby (*Macropus eugenii*); and Monotremata: platypus (*Ornithorhynchus anatinus*) and echidna (*Tachyglossus aculeatus*). Male and female (6 per sex) rats (200-250 g) and mice (8 weeks old) were obtained from Monash Animal House, Monash University, Clayton, Victoria. Liver samples from six adult male tammar wallabies and six adult male brushtail possums were obtained. Two monotreme liver samples were also examined, one from a platypus and another from an echidna. The marsupial and monotreme specimens examined had not been exposed to either fluoroacetate-containing plants and/or any known 1080 baiting program in their recent past. Because of limited resources, only male specimens were used for the Australian native animal study. All animals and samples were handled in accordance with the appropriate ethics and wildlife permits (RMIT MLS9709 for rats; and RMIT MLS9725 for mice; Flora and Fauna permit number 10000360; possum samples were collected by Conservation and Land Management of WA; wallaby samples were collected by the Medical School of the University of Adelaide). Marsupial tissues were stored in liquid nitrogen and all of the other samples were stored at –80°C before use.

Subcellular fractionation of liver

All procedures were carried out at 0-4°C unless otherwise stated. To remove haemoglobin, rodent livers were perfused with 10mM potassium phosphate buffer, pH 7.0, containing 0.15 M KCl, 2 mM DDT, 1 mM EDTA. After perfusion, the livers were removed from the body. Livers of Australian mammals were imported from an interstate source, cut into small pieces and rinsed with the same buffer to remove residual haemoglobin. Liver homogenates were prepared in two volumes (v/w) of the perfusion buffer. Nuclear, mitochondrial, microsomal and cytosolic fractions were prepared by the differential centrifugation method, which was modified from Greenawalt (1974) (Figure 1). Cytochrome P450 (CYP) and succinate dehydrogenase were used as marker enzymes to monitor the contamination of subcellular fractions with either microsomes or mitochondria, respectively.

Assay for defluorination activity

The enzymatic release of free fluoride ion from fluoroacetate was measured according to the procedure previously described by Soiefer and Kostyniak (1984) with several modifications in the standard assay techniques. In brief, the assay was carried out as follows: incubations were performed in a water bath at 37°C for one hour, using an incubation volume of 1.02 mL, which contained 1 mL of the subcellular fraction sample, 10 µL of 1 M sodium fluoroacetate and 10 µL of 0.25 M reduced GSH. The final concentrations of fluoroacetate and reduced GSH were 9.8 mM and 2.45 mM, respectively. The reaction was terminated by the addition of 0.1 mL of 40% (w/v) TCA, and then a few microlitres of 1 M sodium citrate were added to adjust the pH to 4.95-5.05. The fluoride ion concentration was determined by measurement of [F^-] in the pH-adjusted supernatants using a fluoride ion selective electrode (96-09, Orion Research Inc. Boston, MA, USA), according the method of Cowell (1975). Blank samples were prepared by adding 40% TCA to the subcellular fractions before incubating with fluoroacetate and reduced GSH, and then following the same procedure as for the test samples.

A standard calibration curve was made by the same procedure, except that 1 mL of a standard solution of sodium fluoride (0.01, 0.03, 0.1, 0.3, 1, 3, and 10 nmoL/mL) replaced the subcellular fraction in the incubation. A polynomial curve was fitted to the standard curve to assist in accurate interpolation of trace amounts of fluoride ions. The limit of detection for fluoride using this analytical method was 1 nmol/mL, with typical blank control incubations giving readings of 3 ± 0.7 nmol/mL (n=18).

The protein content of subcellular fractions was determined by the method of Lowry *et al.* (1951). The defluorinase activity of each fraction was based on protein content and expressed as nmol F/mg protein/h.

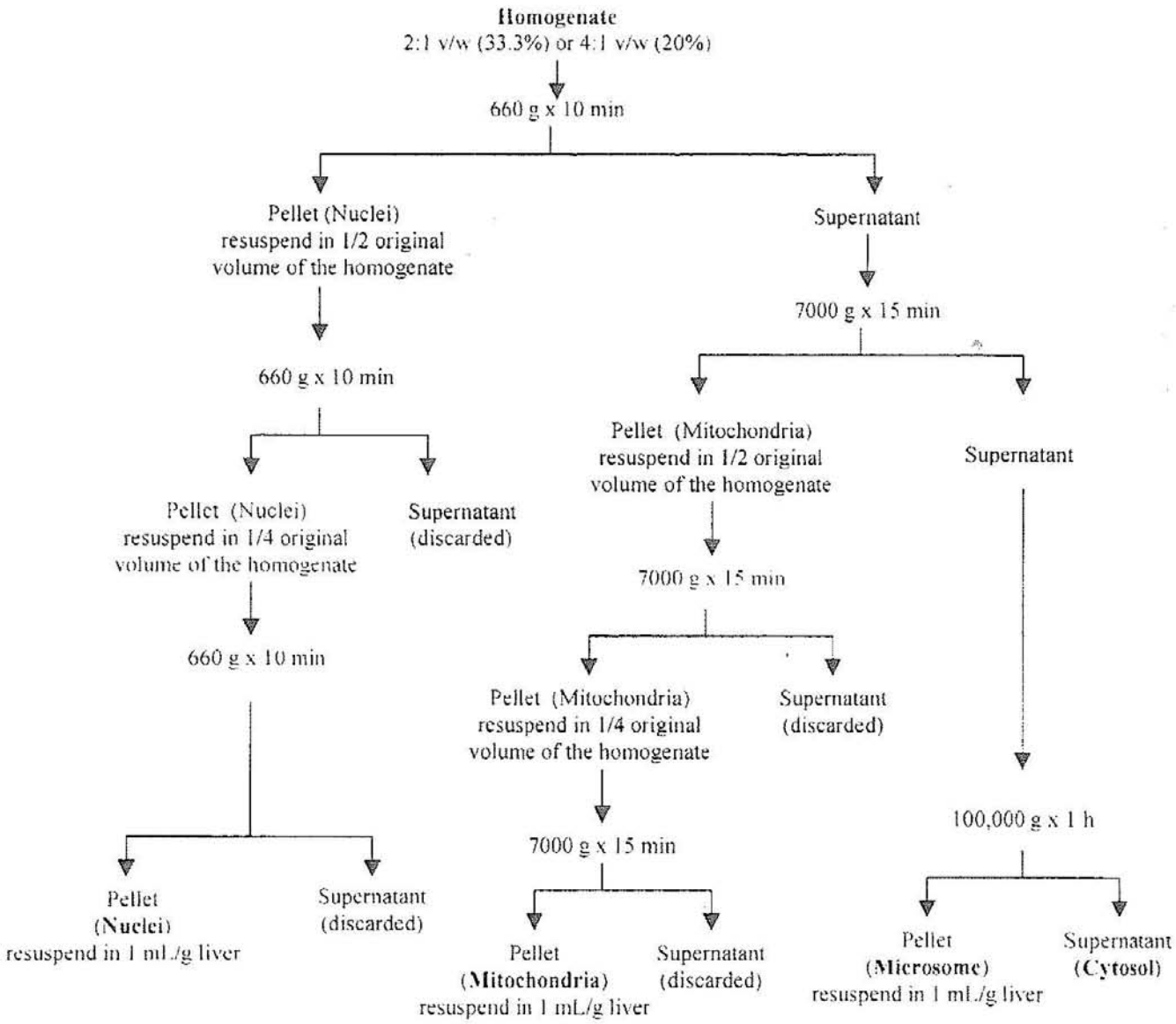

Figure 1. Preparation procedure for the liver subcellular fractions.

Enzyme kinetic studies of FSD in the four species

In order to investigate the defluorination activity of each species, an enzyme kinetic study was performed on liver cytosol fractions obtained from the rat, mouse, brushtail possum and tammar wallaby species. The initial reaction velocities (V_0) were established with concentrations of fluoroacetate at 0, 0.9, 2.3, 4.5, 6.7 and 8.9 mM. The kinetic parameters K_m (Michaelis constant), V_{max} (maximum velocity) and V_{max}/K_m (a combining index used to express the catalytic ability of an enzyme for a particular substrate) were determined. Assays were carried out in triplicate and the non-enzymatic reaction rate was corrected by blank assays in which the protein was denatured prior to addition of substrate.

Data analysis

All data were analysed by one-way analysis of variance and post-hoc Tukey's compromise test, using Super ANOVA statistics software (Abacus Concepts, Inc., Berkeley, CA, USA, 1989).

RESULTS

Subcellular localisation of defluorination activity

The distribution of defluorination activity (% of total subcellular fraction activity) among the subcellular fractions from livers of male rodent, marsupial and monotreme species is shown in Figure 2. Among the liver subcellular fractions, fluoroacetate defluorination activity was consistently higher in the cytosolic fractions of all species investigated, and was significantly different from that in the nucleic, mitochondria, and microsomal fractions ($P<0.05$). The cytosolic fraction of male brushtail possum exhibited a higher defluorination activity (91.5 ± 1.8 % of homogenate activity) than mouse, rat and tammar wallaby (72.2 ± 3.4 %, 72.6 ± 1.4 % and 88.4 ± 1.7 % of homogenate activity, respectively, $P<0.05$). In the single platypus and echidna sample analysed (due to the limited availability of liver samples from these species), it was found that their cytosolic fractions exhibited 78.2% and 82.6% of homogenate defluorination activity, respectively (Figure 2). The cytosolic fractions of both platypus and echidna samples showed a higher defluorination activity than marsupials, expressed as per gram liver per hour.

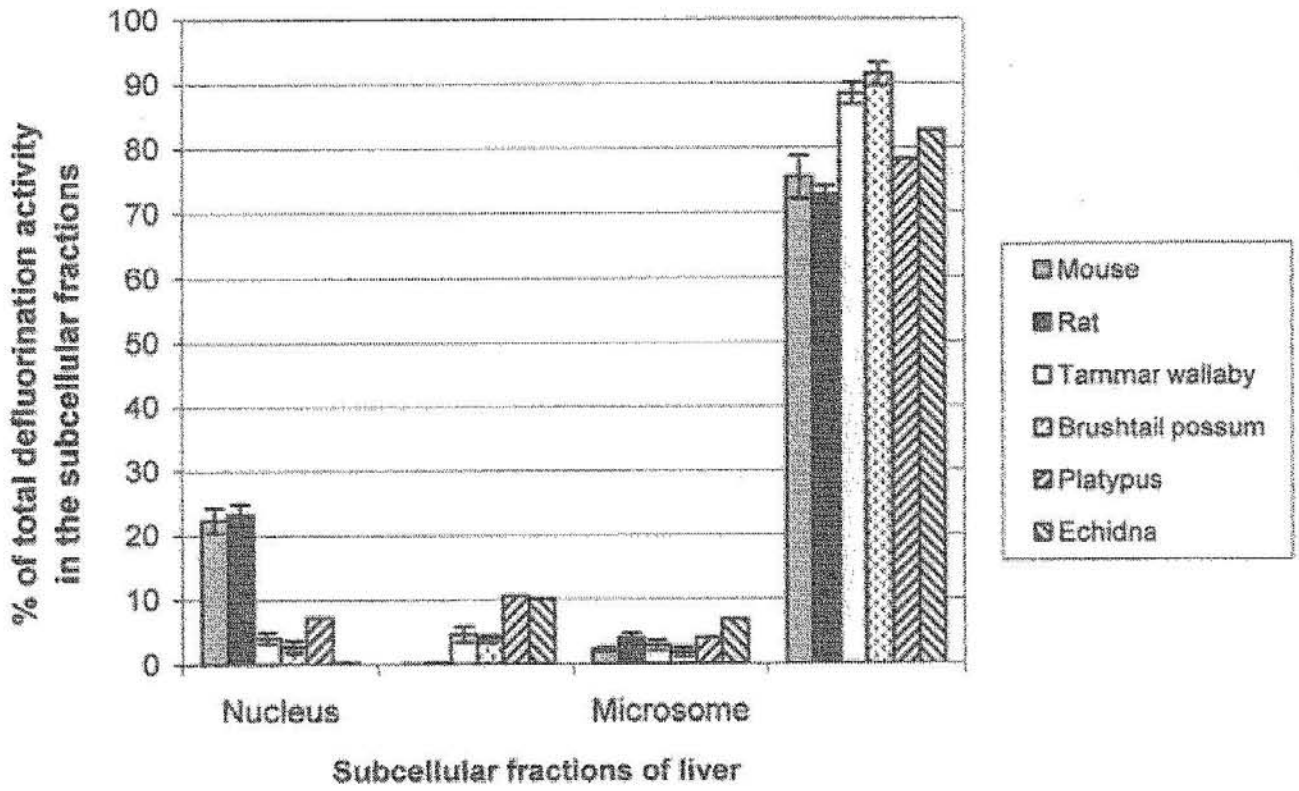

Figure 2. Subcellular localisation of defluorination activity in the livers of rodents, marsupials and monotremes species (male adults, mean ± SE, n=6 in rodent and marsupial species, n=1 in monotreme species).

Cytochrome P450 and succinate dehydrogenase are marker enzymes of microsome and mitochondria respectively. In this work, their activities were less than 3% in all other subcellular fractions, indicating that contamination from mitochondria and microsomes had been minimised by the experimental procedures.

Comparison of defluorination activity in rodent and marsupial species

A comparison of the defluorination activity in rodent and marsupial species is shown in Table 1. In all species, the highest defluorination activity was obtained in the cytosolic fraction, and this was the only fraction which showed an increase in specific activity over that of the original homogenate. The rat, possum and mouse had a significantly higher cytosolic activity compared with the wallaby ($P<0.05$). In addition, significantly higher mitochondrial activities were found in possum and wallaby compared to the mouse ($P<0.05$). It was also noted that some defluorination activity existed in the nuclear fraction of livers in all of the species. Platypus and echidna also showed the highest defluorination activities in the cytosolic fraction of livers (Table 2). Although only single samples were available from each monotreme species, the homogenate and cytosolic defluorination activities in the echidna may be greater than the other species when standardised for protein content.

The total fluoroacetate detoxication capacities of rodent, marsupial, and monotreme species were also compared. Platypus and echidna showed the highest defluorination capacities (1.74 and 1.71 μmol F/g liver/h, respectively). Rat and mouse showed significantly higher defluorination capacities (1.34 ± 0.10, 1.08 ± 0.03 μmol F/g liver/h) than possum and wallaby (0.71 ± 0.07, 0.76 ± 0.11 μmol F/g liver/h) ($n=6$, $P<0.05$).

Comparison of sex-differences in defluorination activity

The abilities of male and female rats and mice to defluorinate fluoroacetate are compared in Table 1. There is no significant statistical difference in the defluorination activity between the sexes in the laboratory rodents examined ($P>0.05$).

Comparison of defluorination enzyme kinetics

Kinetic parameters for fluoroacetate defluorination activity were determined with rat, mouse, brushtail possum and tammar wallaby liver cytosolic fractions (Table 3). Eight male rats and six male individuals in all other species have been used for these enzyme kinetic studies. The rat had a significantly lower K_m than the brushtail possum, tammar wallaby and mouse ($P<0.05$). There were also significant species differences in V_{max}, with the mouse having the highest V_{max} followed by the rat, wallaby and possum, in order of decreasing maximum catalytic velocity. The V_{max}/K_m values for the mouse and rat were significantly higher than those for possum and wallaby ($P<0.05$).

DISCUSSION

Australian native mammals living outside the range of fluoroacetate-containing plants were sampled and used in the study to localise the fluoroacetate detoxication enzymes in the liver subcellular fractions of Australian mammals. In this investigation, we used a modified method of fluoride ion determination to study the subcellular distribution of liver defluorination activity *in vitro* in the representative rodent species (mouse and rat), Marsupialia (brushtail possum and tammar wallaby) and Monotremata (platypus and echidna). Fluoroacetate at 9.8 mM was chosen as the substrate

Table 1. Comparison of defluorination activity in liver subcellular fractions of rodent and marsupial species (adults, mean ± SE, n=6 per species).

Subcellular fraction	Defluorination activity (nmol F⁻/mg protein/h)					
	Mouse		Rat		Brushtail possum	Tammar wallaby
	Male	Female[#]	Male	Female[#]	Male	Male
Homogenate	5.75 ± 0.13[b]	6.41 ± 0.35	7.54 ± 0.39[c]	8.05 ± 0.41	4.54 ± 0.47[b]	3.18 ± 0.30[a]
Nuclei	3.26 ± 0.18[b]	3.59 ± 0.17	5.02 ± 0.31[c]	6.03 ± 0.40	3.66 ± 0.20[b]	1.15 ± 0.38[a]
Mitochondria	0.11 ± 0.11[a]	ND	2.12 ± 0.59[a,b]	1.86 ± 0.10	3.81 ± 0.44[b]	2.71± 0.77[b]
Microsomes	1.12 ± 0.17	2.60 ± 0.71	2.04 ± 0.40	1.24 ± 0.25	1.94 ± 0.05	1.85 ± 0.28
Cytosol	13.82± 0.77[b]	18.82 ± 2.35	15.39 ± 0.76[b]	17.25 ± 0.76	13.46 ± 0.76[b]	7.46 ± 0.71[a]

[#] Female activities were not significantly different from male values.
ND: no detectable level of activity.
[a b c] Within each fraction, the male values with the same letter are not significantly different (P<0.05).

Table 2. The subcellular distribution of defluorination activity in platypus and echidna (male adult, n=1 per species).

Subcellular fraction	Defluorination activity (nmol F⁻/mg protein/h)	
	Platypus	Echidna
Homogenate	7.27	10.63
Nuclei	1.74	0.51
Mitochondria	2.79	2.05
Microsome	2.40	2.40
Cytosol	14.35	20.48

concentration because it is within the linear range of the reaction rate (0-19.6 mM) and because it has been used in previous studies (Kostyniak *et al.* 1978; Soiefer and Kostyniak 1983, 1984), making the present data more readily comparable with previous reports in the literature.

The results suggest that the defluorination of fluoroacetate is mainly localised in the cytosolic fraction of the liver (Table 1; Figure 2), a finding which is in agreement with those of Kostyniak *et al.* (1978). In rat liver, the cytosolic fraction exhibited the highest activity, while the nuclear fraction contained a low level of defluorination activity (Table 1), which is also similar to previous reports (Kostyniak *et al.* 1978). However, the mitochondrial fraction exhibited only limited defluorination activity in our study, which is different from previous studies where the defluorination activity was reported to be as high as that of the nuclear fraction (Kostyniak *et al.* 1978). This difference may be due to the methodological differences in the preparation of subcellular liver fractions: the present study incorporated three washes of both nuclear and mitochondrial fractions to ensure the removal of cytosolic contamination. Consequently, the results from this study reveal the enzymatic nature of the fluoroacetate defluorination reaction, and will provide information for further characterisation of the fluoroacetate detoxication enzymes.

The comparative study of subcellular distribution of liver defluorination activity in the four species suggests that there are clear species-related differences in defluorination activity. The defluorination activity (nmol F⁻/mg protein/h) in cytosolic fractions from the four species were as follows: rat > mouse > brushtail possum > tammar wallaby, with the wallaby having a significantly lower activity than the other species (Table 1). Marsupial metabolic rates are usually lower than those of equivalent-sized eutherians (McNab 1980), and although FSD activities display this trend, there were no significant differences (P>0.05) in FSD activities between rodent species and the brushtail possum. As this trend is the reverse of the known fluoroacetate sensitivities of these species, differences in the ability to defluorinate fluoroacetate are unlikely to account for the differing sensitivities of the particular species to fluoroacetate. The cytosol FSD activity of the brushtail possum was similar to the range of defluorination activity previously determined in the liver acetone powder fraction of the same species (Mead *et al.* 1979). As already noted in the rat, the defluorination activity was consistently found in the nuclear fraction for all species, and although it contributed only a small percentage of the total amount of homogenate defluorination activity, it may nevertheless represent a significant cellular compartment for defluorination.

However, the marsupials have considerably more defluorination activity in the mitochondrial fraction, where the highest activity was more than 30-fold of the FSD activity in the mouse mitochondrial fraction (p<0.05) and nearly twice that of the rats. It is known that mitochondria have a high catalytic efficiency for electrophiles and electrophilic xenobiotics (Morgenstern and Depierre 1987), so it is quite possible that some mitochondrial enzymes are also involved in defluorination. It has been reported that GSH significantly stimulates defluorination activity in the liver and protects mitochondrial enzymes from fluorocitrate, therefore the depletion of liver GSH is associated with a significantly greater elevation of plasma citrate levels in animals (Mead *et al.* 1979, 1985; Kostyniak *et al.* 1978). Clearly, further investigations are necessary to determine the possible role that mitochondria may play in the detoxication/defluorination of fluoroacetate.

Table 3. Kinetic parameters for defluorination activity of fluoroacetate in the liver cytosol of the mouse, rat, possum and wallaby (male adults, mean ± SE, n=8 in the rat, n=6 in the other species).

Species	K_m (mM)	V_{max} (nmol F^-/mg protein/h)	V_{max}/K_m
Mouse	8.97 ± 1.98^b	73.99 ± 10.28^c	8.53 ± 0.75^b
Rat	3.50 ± 0.42^a	26.51 ± 1.44^b	8.12 ± 0.71^b
Brushtail Possum	$6.71 \pm 0.81^{a,b}$	8.48 ± 1.04^a	1.33 ± 0.30^a
Tammar Wallaby	8.80 ± 1.90^b	9.39 ± 1.20^a	1.11 ± 0.11^a

$^{a\,b\,c}$ For each kinetic parameter, the values with the same letter are not significantly different ($P<0.05$).

The platypus and echidna are unique monotreme species and knowledge about their metabolism is limited. Consequently, it was of great interest to investigate whether these mammals can detoxify fluoroacetate. The platypus mainly lives in aquatic habitats, whilst the echidna lives on land, so it may be presumed that the echidna would be more likely to be exposed to fluoroacetate-containing plants, or possibly consume ants and termites that have come in contact with fluoroacetate-containing vegetation. However, this has not been confirmed although it has been suggested that the dasyurid (*Phascogale calura*), an insectivorous marsupial, may acquire a high tolerance to fluoroacetate through ingestion of insects which feed on the toxic plants (Kitchener 1981, Twigg and King 1991). Our study demonstrated that the echidna sample exhibited a higher defluorination activity in liver cytosol (20.48 nmol/mg protein/h) than that of platypus (14.35 nmol/mg protein/h) and the samples obtained from males of the other species which were investigated (Table 2). Since we were unable to obtain more platypus or echidna liver samples, it was not possible to statistically compare the defluorination activity of monotremes with the other mammalian species. Nevertheless, it is apparent that they appear to have the same subcellular distribution profile as that seen for the mammalian species. However, the possibility of an innate tolerance to fluoroacetate in these unadapted animals, which have not been exposed to fluoroacetate-containing plants, can not be discounted.

Sex differences have been noted in the activity of P450 and GST liver biotransformation enzymes in rodent and marsupial species (Tanaka 1999, Bonate 1991). However, no previous studies have investigated sex-related differences in FSD activity. In the present study, there was no statistical significant difference in the FSD activity between the sexes in the laboratory rodents examined (Table 1). As there are major limitations in obtaining sufficient marsupial samples, only male samples were available and it was not possible to determine whether there are any sex-related differences in FSD activity. This aspect remains to be investigated when female samples are available.

The enzyme kinetic parameters V_{max} and K_m were determined in the liver cytosolic fraction of rodent and marsupial species to investigate whether there is any species difference in defluorination activity. V_{max} is the constant catalytic rate that is reached when the enzyme is completely saturated with substrate. K_m is equal to the concentration of substrate (denoted '[S]') at which the reaction velocity is half of V_{max}, and it is also indicative of the affinity between the enzyme and substrate. V_{max}/K_m indicates the saturation ability with a high value indicating greater enzyme affinity for the substrate. The enzyme kinetic data shows that there were clear differences between species in FSD activity (Table 3). Mice exhibit the same K_m value as brushtail possum and tammar wallaby, but a significantly higher V_{max} and V_{max}/K_m. Rats exhibited significantly lower K_m and higher V_{max} and V_{max}/K_m values than brushtail possum and tammar wallaby. This means that rodent species have higher fluoroacetate detoxication capabilities compared with the two unadapted Australian marsupials. The LD_{50} of fluoroacetate in rodents and marsupials suggests that marsupials are more resistant to fluoroacetate. However, this study showed significant lower affinity between marsupial FSD and fluoroacetate, compared with rodent species. Obviously, fluoroacetate detoxication is not the main mechanism of fluoroacetate tolerance of Australian marsupials.

In this study, the rodents were fed with a non-fluoroacetate diet, and all of the marsupials were collected from regions without fluoroacetate vegetation. Thus, we cannot draw conclusions about the effect of a fluoroacetate-containing diet on the enzymatic nature of FSD. It has been reported that marsupials are disadvantaged by having lower rates of oxidative xenobiotic metabolism than their eutherian counterparts have (McManus and Ilett 1976, 1977). The present study shows that the Australian marsupials living outside the range of fluoroacetate-containing plants have a lower fluoroacetate detoxication ability than rodents, which suggests that marsupials may also be further disadvantaged in their metabolic detoxication of xenobiotics. Therefore, in order to survive in regions of fluoroacetate-containing plants (mainly western and northern regions of Australia), some genetic or enzymatic adaptation of marsupials to their environment could be expected as an inevitable result of evolution.

Current evidence suggests that the tolerance to fluoroacetate among the Australian mammals (and other animals) living within the range of fluoroacetate-containing plants is not related to the ability to defluorinate fluoroacetate, but rather, it is the likely result of differences in the effect of fluorocitrate on citrate transport mechanisms and/or the effect of fluoroacetate on the Krebs cycle enzymes.

In eutherian mammals, defluorination occurs mainly in the liver by a GSH-dependent mechanism. Defluorination of fluoroacetate is a ubiquitous detoxication reaction but the mechanism is still unclear. The isolation and characterisation of the fluoroacetate defluorinating system in mammalian liver is the key to determining whether the defluorination reaction is catalysed by a unique GST, as hypothesised.

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