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Metabolism and Defluorination of Fluoroacetate in the Brush-tailed Possum (*Trichosurus vulpecula*)

R. J. Mead,[A] *A. J. Oliver*[B] *and D. R. King*[B]

[A] Department of Biochemistry, University of Western Australia, Nedlands, W.A. 6009.
[B] Agricultural Protection Board of Western Australia, Dawson Avenue, Forrestfield, W.A. 6058.

Abstract

The brush-tailed possum (*T. vulpecula*) from Western Australia was found to be nearly 150 times more resistant to fluoroacetate intoxication *in vivo* than the same species from South Australia. Acetone powder preparations from the liver of animals from both populations showed similar abilities to convert fluoroacetate into fluorocitrate. Aconitate hydratase activity in liver preparations from both Western Australian and South Australian animals was similarly and competitively inhibited by fluorocitrate. Both animals were capable of defluorinating fluoroacetate at similar rates by a glutathione-dependent enzymic mechanism resulting in the formation of free fluoride ion and *S*-carboxymethylcysteine. Glutathione was also capable of partial protection against the toxic effects of fluoroacetate *in vitro* by a further unelucidated mechanism.

Introduction

Fluoroacetate is thought to be highly toxic to mammals due to its conversion within the animal to fluoroacetyl coenzyme A and thence to fluorocitrate (Fig. 1). The latter compound inhibits aconitate hydratase [citrate (isocitrate) hydrolase, EC 4.2.1.3] and blocks the Kreb's cycle at the citrate stage (Morrison and Peters 1954). This results in citrate accumulation in the tissues and increases in the concentration of citrate in the plasma.

In the course of our investigation of the toxicity of sodium monofluoroacetate (compound 1080) to Australian native fauna, it became apparent that some species living within the range of fluoroacetate-containing-plants possessed an unusually high tolerance (Oliver *et al.* 1977; King *et al.* 1978), while corresponding species living in different areas were far more susceptible to the toxin.

This difference is exemplified by comparison of the brush-tailed possum (*Trichosurus vulpecula*) from south-western Australia and eastern Australia [LD_{50} south-western Australia *c.* 100 mg/kg (Oliver *et al.* 1977); LD_{50} eastern Australia 0·68 mg/kg, (J. C. McIlroy, personal communication)].

Large differences in fluoroacetate tolerance could be expected to result from (1) differences in the ease of conversion of fluoroacetetate to fluorocitrate, (2) differences in the sensitivity of aconitate hydratase to fluorocitrate, or (3) differences in the metabolism of fluoroacetate. This paper presents the results of a preliminary study of these possibilities.

Materials and Methods

Animals

Possums were collected in the field from several localities in the south-west of Western Australia and in the Mt Lofty Ranges, South Australia. When necessary, the animals were housed in metabolism cages fitted with plastic containers to facilitate urine collection.

Acetone Powder Preparations

Animals were killed by cardiac puncture and exsanguination. Livers were rapidly excised and homogenized in a 10-fold excess of AR acetone (−5°C). The protein precipitate was collected by filtration under vacuum and dried by suction for 30 min at 2°C.

The dried powder, 5 g, was gently stirred for 2 h with 10 ml of 0·1 M potassium phosphate buffer, pH 7·6. The mixture was centrifuged in a bench centrifuge at 2°C to remove undissolved material and the supernatant was dialysed for 36 h at 2°C against three changes of 0·1 M potassium phosphate buffer, pH 7·6. The resultant solution was used as the enzyme source for all *in vitro* studies.

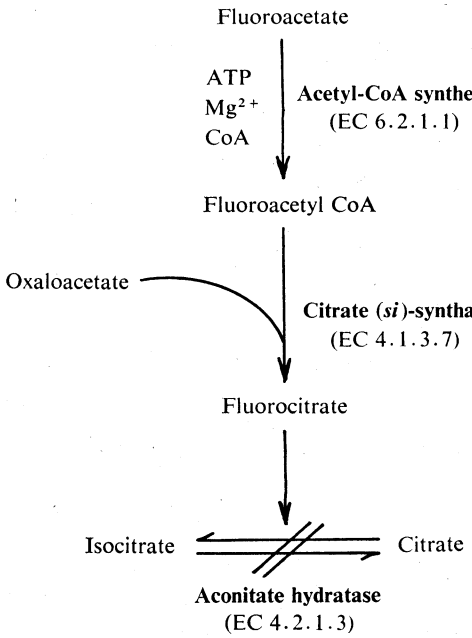

Fig. 1. Pathway of conversion of fluoroacetate into fluorocitrate and inhibition of aconitate hydratase.

Citrate Estimation

Citrate concentration was determined in both plasma and enzyme incubation mixtures by the method of Camp and Farmer (1967).

Fluoride Estimation

Fluoride ion concentration was determined using an Orion specific ion electrode.

Protein Estimation

Protein concentration was determined by the method of Pesce and Strande (1973).

Gas–Liquid Chromatography—Mass Spectrometry of Amino Acids

Enzyme incubation mixtures were deproteinized by the addition of 1·0 ml (15% (w/v) trichloroacetic acid (TCA). Urine samples were processed without deproteinization. 1 ml of either urine

or incubation mixture supernatant was adjusted to pH 2·0–2·5 by the addition of approximately 0·5 ml of a 50% solution of acetic acid containing 5·0 mg $SnCl_2$ per 100 ml.

The resultant solutions were passed through a small ion-exchange column (3 by 1 cm) containing Dowex 50W (H^+) 12% cross-linked resin, dry mesh 50–100, at a rate of about one drop per 5 s. After washing the column with 1 ml of deionized water, the amino acid fraction was eluted using 3 ml 2 M NH_4OH passed through the column at about one drop per second.

The eluates were evaporated to dryness in a rotary evaporator and the amino acids were converted to their methyl-*N*-trifluoroacetyl derivatives by the method of Dabre and Islam (1968). G.l.c.–mass spectrometric analysis was performed on the derivatized amino acid fraction using a Varian Aerograph 2700 gas chromatograph (stationary phase support chromosorb-W; liquid phase 10% OV-17). The temperature was maintained at 100°C for 10 min and was increased to 330°C at 10°/min. The dimethyl-*N*-trifluoroacetyl derivative of *S*-carboxymethylcysteine had a retention time of 22 min in this system. The gas chromatograph was linked to a Varian-mat 311 mass spectrometer and a Varian SS100 computer system for mass spectrometric analysis.

Amino Acid Analysis

The concentration of urinary *S*-carboxymethylcysteine was determined using the 4 lithium buffer system on an LKB automated amino acid analyser model 3201, after determining the elution characteristics of *S*-carboxymethylcysteine with authentic solutions of known concentration.

Biochemicals

Fluorocitrate. Barium fluorocitrate (Calbiochem San Diego, California) was dissolved in a minimal quantity of 0·1 M HCl and the barium precipitated by the stoichiometric addition of Na_2SO_4. The supernatant, containing sodium fluorocitrate, was neutralized with solid Na_2CO_3 and adjusted to known volume with 0·1 M potassium phosphate buffer, pH 7·6.

Trisodium isocitrate, cis-oxaloacetic acid, adenosine 5′-triphosphate (ATP), coenzyme A (lithium salt), reduced glutathione, authentic *S*-carboxymethylcysteine and Dowex 50W resin were all obtained from Sigma Chemical Co., St Louis, Missouri. Commercial grade Tenate brand sodium fluoroacetate, obtained from Rentokil Laboratories Ltd, was used in all dosing experiments. Its purity was determined to be 92% with respect to sodium fluoroacetate as described earlier (King *et al.* 1978). For all *in vitro* studies sodium fluoroacetate obtained from B.D.H. Ltd (Poole, England) was used.

Results

Plasma Citrate and Fluoride Levels

The changes in plasma citrate concentrations of Western Australian and South Australian brush-tailed possums in response to various doses of fluoroacetate are shown in Fig. 2. A dose of 100 mg/kg to a Western Australian possum was needed to invoke a response similar to that produced in a South Australian possum dosed at 0·5 mg/kg. This suggested a more complete inhibition of aconitate hydratase in the South Australian animals.

It was also found that the Western Australian possum appeared to defluorinate fluoroacetate or one of its metabolites as indicated by elevation of plasma fluoride ion levels post-dosing (Fig. 3). It was not possible to determine whether the South Australian possum was also capable of defluorination *in vivo*, as dosing with quantities of fluoroacetate sufficient to detect changes in plasma fluoride ion concentration resulted in the early death of the animal.

Aconitate Hydratase Sensitivity

The activity of aconitate hydratase in the presence and absence of fluorocitrate as expressed in Lineweaver–Burk plots for both Western Australian and South Australian possums is shown in Fig. 4. Preparations from both Western Australian and South Australian possums yielded a K_m value of 0·39 mM with respect to L-iso-

citrate as determined by the weighted least-squares method of Wilkinson (1961), and both preparations showed classical competitive inhibition with fluorocitrate (K_i Western Australian, 0·044 mM; K_i South Australian, 0·032 mM).

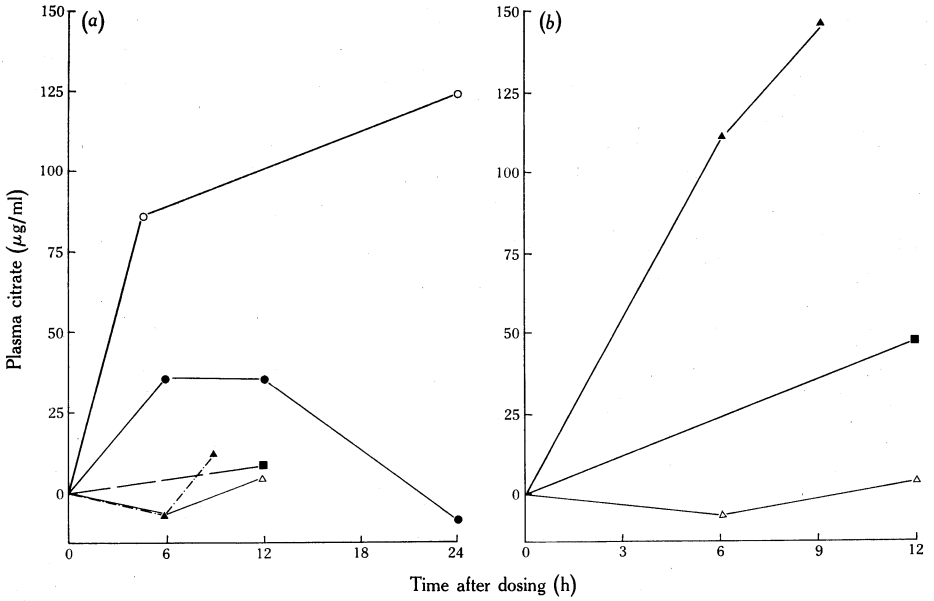

Fig. 2. Changes in plasma citrate concentration in (*a*) Western Australian and (*b*) South Australian possums after dosing with sodium fluoroacetate. Results are expressed as increases above base-line levels. Dosages: ○ 100 mg/kg (n = 2); ● 20 mg/kg (n = 6); ▲ 0·5 mg/kg (n = 2); ■ 0·2 mg/kg (n = 3); △ undosed controls (n = 2).

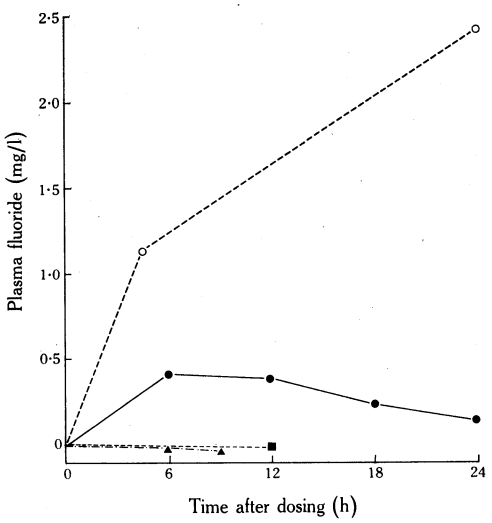

Fig. 3. Changes in plasma fluoride concentration in Western Australian possums after dosing with sodium fluoroacetate. Results are expressed as increases above base-line levels. Dosages:
○ 100 mg/kg (n = 2);
● 20 mg/kg (n = 6);
▲ 0·5 mg/kg (n = 2);
■ 0·2 mg/kg (n = 2).

Conversion of Fluoroacetate to Fluorocitrate

Acetone powder preparations of possum liver were incubated with fluoroacetate, ATP, Mg^{2+}, coenzyme A, and oxaloacetate for 45 min. Isocitrate was then added

and the rate of its conversion into citrate by aconitate hydratase present in the preparations was monitored over a short period to assess the extent of fluorocitrate formation.

Both South Australian and Western Australian possums were capable of considerable fluorocitrate synthesis within the 45-min incubation period (Table 1). In each group, decreasing the length of the incubation period to 30 min resulted in an almost identical degree of inhibition of aconitate hydratase. Decreasing the incubation time to 15 min still allowed sufficient time for considerable fluorocitrate synthesis and significant aconitate hydratase inhibition to occur. In incubation mixtures containing 5 mM fluoroacetate, incubated for 15 min prior to isocitrate addition, aconitate hydratase activity in South Australian preparations was inhibited by 51% while aconitate hydratase activity in Western Australian preparations was inhibited by 41% (cf. 84 and 87% respectively with a 45-min incubation period—see Table 1).

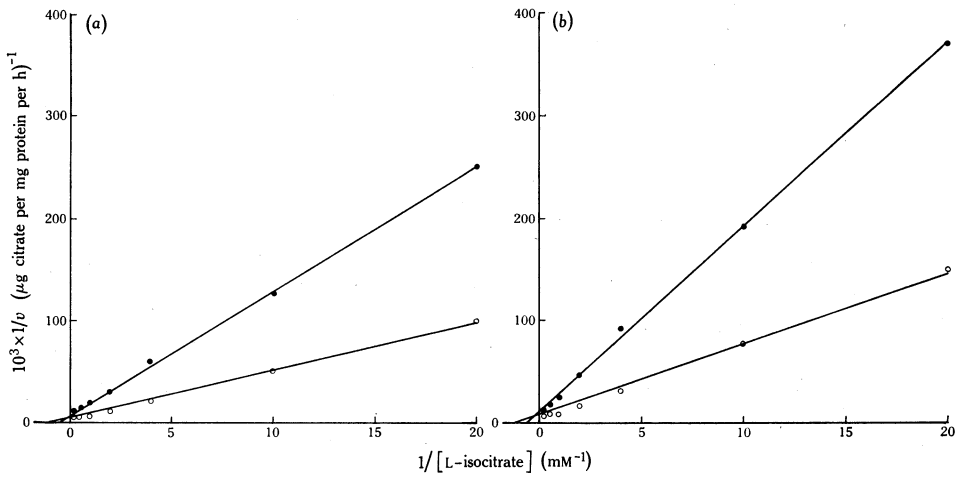

Fig. 4. Lineweaver–Burk plots of aconitate hydratase activity in liver acetone powder preparations of (*a*) Western Australian and (*b*) South Australian possums with and without fluorocitrate. Aliquots of enzyme preparations containing between 1 and 2 mg protein were pre-incubated for 15 min at 37°C with or without L-fluorocitrate to a final incubation concentration of 0·13 mM. Isocitrate was then added and the reaction allowed to proceed for 30 min at 37°C. Reaction was stopped by the addition of 1·0 ml 15% (w/v) TCA and citrate was determined. (Incubation volume 1·0 ml, 0·1 M potassium phosphate buffer, pH 7·6.) ○ Without fluorocitrate. ● 0·13 mM L-fluorocitrate.

Defluorination

To confirm the apparent defluorination obtained in Western Australian possums *in vivo* (Fig. 4), liver acetone powder preparations of Western Australian and South Australian possums were incubated with fluoroacetate and the free fluoride ion concentration of the incubation mixture was monitored with time. Significant defluorination occurred only if glutathione (GSH) was added to the incubation mixture (Table 2). Fluoroacetate and fluoroacetyl CoA (synthesized *in vitro* by the acetone powder preparations) proved to be effective substrates for defluorination in the presence of GSH (Table 2). It is apparent, however, that activation of fluoroacetate to fluoroacetyl CoA is not a necessary prerequisite for defluorination.

Table 1. Synthesis of fluorocitrate from added fluoroacetate by liver acetone powder preparations from Western Australian and South Australian possums as determined by aconitate hydratase inhibition

The complete incubation mixture contained enzyme preparation (Enz) (4–6 mg protein), fluoroacetate (FAc) (2·5 or 5 mM), ATP (5 mM), coenzyme A (CoA) (0·5 mM), $MgCl_2$ (4 mM), and oxaloacetate (OAc) (5 mM). Reaction was allowed to proceed for 45 min at 37°C prior to the addition of isocitrate to an incubation mixture concentration of 5 mM. 1·0 ml 15% (w/v) TCA was added to individual tubes after a further 2, 4, 8 and 12 min incubation at 37°C and the rate of citrate production was determined (incubation volume 1·0 ml, 0·1 M potassium phosphate buffer, pH 7·6)

Additives	Aconitate hydratase activity (μg citrate per mg protein per h) in:					
	Western Australian possums			South Australian possums		
	Animal 1	Animal 2	Animal 3	Animal 1	Animal 2	Animal 3
$[Enz]_{45min}$+isocitrate	213	155	187	236	190	70
$[Enz+CoA+ATP+Mg^{2+}]_{45min}$ +isocitrate	225	150	186	240	195	68
$[Enz+2{\cdot}5$ mM $FAc]_{45min}$ +isocitrate	225	—	—	242	—	—
$[Enz+2{\cdot}5$ mM $FAc+ATP+CoA+Mg^{2+}]_{45min}$ +isocitrate	220	—	—	243	—	—
$[Enz+2{\cdot}5$ mM $FAc+ATP+CoA+Mg^{2+}+OAc]_{45min}$ +isocitrate	113	—	—	132	—	—
$[Enz+5$ mM $FAc]_{45min}$ +isocitrate	—	153	190	—	198	71
$[Enz+5$ mM $FAc+CoA+ATP+Mg^{2+}]_{45min}$ +isocitrate	—	155	192	—	198	69
$[Enz+5$ mM $FAc+CoA+ATP+Mg^{2+}+OAc]_{45min}$ +isocitrate	—	19·5	27·6	—	31·5	9
Percentage inhibition	49	87	85	45	84	87

The addition of oxaloacetate to convert fluoroacetyl CoA to fluorocitrate resulted in a considerable reduction in the rate of defluorination (Table 2). No defluorination occurred in the absence of enzyme or in the presence of a trichloroacetic acid-inactivated enzyme preparation during a 48-h incubation. South Australian possum liver preparations proved to be similarly capable of defluorinating fluoroacetate (Table 2).

A small amount of defluorination was consistently obtained when coenzyme A was incubated with fluoroacetate or with fluoroacetate, Mg^{2+} and ATP in the absence of GSH. The presence of a sulfhydryl group in both coenzyme A and GSH implicated the sulfhydryl group in the defluorination mechanism.

Table 2. *In vitro* defluorination of fluoroacetate and fluoroacetate derivatives by liver acetone powder preparations of Western Australian and South Australian possums

The complete incubation mixture contained enzyme preparation (10–15 mg protein), fluoroacetate (FAc) (5 mM), ATP (5 mM), $MgCl_2$ (4 mM), oxaloacetate (OAc) (10 mM), CoA (0·5 mM), and GSH (5 mM). Tubes were incubated at 37°C for 0, 30, 60 and 120 min at pH 7·6 (0·1 M potassium phosphate buffer) in an incubation volume of 1·0 ml. Reaction was stopped by the addition of 1·0 ml 15% (w/v) TCA and the rate of defluorination was determined by free fluoride ion measurement. Values in parentheses are the number of determinations

Substrates	F^- released (μg per g protein per h) W.A. possums	S.A. possums
FAc+GSH	139±24 (4)	130±20 (4)
FAc	0	
FAc+ATP+Mg^{2+}	0	
FAc+ATP+Mg^{2+}+GSH	114	
FAc+ATP+Mg^{2+}+CoA	11	
FAc+ATP+Mg^{2+}+CoA+GSH	114	
FAc+ATP+Mg^{2+}+CoA+OAc	0	
FAc+ATP+Mg^{2+}+CoA+OAc+GSH	69	

With 5 mM fluoroacetate and 5 mM GSH in an incubation volume of 1·0 ml, the rate of *in vitro* defluorination was linear for 2 h. Increasing the concentration of GSH to 10 and 15 mM did not increase the initial rate of defluorination but the reaction remained linear for a longer period. The total amount of fluoride released was greater after 16 h with 10 mM GSH (F^- released = 1448 μg/g protein) and 15 mM GSH (F^- released = 1674 μg/g protein) than had occurred with 5 mM GSH (F^- released = 990 μg/g protein).

To determine the mechanism of defluorination, an aliquot of acetone powder preparation was incubated with fluoroacetate (5 mM) and GSH (10 mM) in a final volume of 1·0 ml for 24 h. Isolation of the amino acids present in the incubation mixture and their analysis by g.l.c.–mass spectrometry as described in Materials and Methods established the presence of *S*-carboxymethylcysteine. As assessed by g.l.c.–mass spectrometry, *S*-carboxymethylcysteine was not produced in incubation mixtures containing inactivated enzyme preparation or from which fluoroacetate was omitted.

To confirm the *in vivo* production of this metabolite, a Western Australian possum was dosed with 50 mg/kg sodium fluoroacetate. Urine samples collected prior to dosing and in the interval 17–24 h post-dosing were examined for the presence of *S*-carboxymethylcysteine by g.l.c.–mass spectrometry. No *S*-carboxymethylcysteine could be detected prior to dosing but its presence was confirmed in the post-dosing sample.

The mass spectra of the dimethyl-*N*-trifluoroacetyl derivatives of authentic *S*-carboxymethylcysteine, the amino acid produced *in vitro* by the enzyme-catalysed reaction of fluoroacetate and GSH, and the amino acid isolated from the urine were identical: *m/e* M^+ 303; 184, loss of esterified carboxyl groups; 119, fragmentation between α and β carbons of the cysteine; 158, formation of a keten at the carboxyl group of the cysteine.

The presence of *S*-carboxymethylcysteine was further confirmed by chromatography on an automated amino acid analyser where it was found to elute close to aspartate. Its concentration in the post-dosing urine was calculated to be 2100 μmol/l, but the extent to which the mercapturic acid (*S*-carboxymethylcysteine-*N*-acetate) was present was not determined.

Protective Effect of Glutathione

Acetone powder preparations of possum liver were incubated with fluoroacetate, ATP, Mg^{2+}, coenzyme A, oxaloacetate and GSH for 45 min. Isocitrate was then added and the rate of its conversion into citrate by aconitate hydratase present in the preparations was monitored to assess the extent of fluorocitrate formation as described above. Aconitate hydratase activity was less inhibited in the presence of GSH than in its absence in both Western Australian and South Australian possum liver preparations (Fig. 5). This effect of GSH was not solely a result of its stimulation of the rate of defluorination of fluoroacetate as only 0·18% of added fluoroacetate was defluorinated by the enzyme preparation from Western Australian possums during the incubation period, as assessed by free fluoride ion measurement.

Discussion

In vivo defluorination of fluoroacetate or its metabolites has previously been demonstrated in the laboratory rat (Peters *et al.* 1972; Smith *et al.* 1977), in red and grey kangaroos (Oliver *et al.* 1977) and in Australian possums and bush rats (King *et al.* 1978). Substantial detoxification of fluoroacetate by defluorination has also been reported in soil bacteria (Horiuchi 1960; Goldman 1965; Kelly 1965). The mechanism of bacterial defluorination has been shown to involve an enzymic cleavage of fluoroacetate to yield fluoride ion and glycolate (Goldman 1965).

In vivo defluorination of fluoroacetate has also been demonstrated in peanuts (Preuss and Weinstein 1969) and in lettuce (Ward and Huskisson 1972). As far as we are aware, substantial *in vitro* defluorination of fluoroacetate by animal tissues has not previously been reported.

The defluorination process described in this paper appears to involve an enzyme of the glutathione-*S*-transferase type. Such enzymes have been implicated in the detoxification of aryl (Booth *et al.* 1961), aralkyl (Boyland and Chasseaud 1969), epoxide (Boyland and Williams 1965; Fjellstedt *et al.* 1973) and alkyl (Johnson 1966) compounds by converting them into glutathione conjugates. Dehalogenation of

chloroacetic acid in mice *in vivo* has been demonstrated (Yllner 1971) and concomittant excretion of *S*-carboxymethylcysteine occurs in the urine. Bromoacetic acid is also metabolized to *S*-carboxymethylglutathione in cockroaches (Bettini and Boccacci 1958).

It now appears from the data presented in this paper that the C–F bond of fluoroacetate, more stable than the C–Cl and C–Br bonds of chloroacetic and bromoacetic acids respectively, is similarly cleaved by animal tissues. The defluorination appears to result from a nucleophilic attack on the β-carbon of fluoroacetate with resultant release of free fluoride and the formation of *S*-carboxymethylglutathione. The latter is apparently cleaved into its component amino acids and *S*-carboxymethylcysteine is excreted in the urine (Fig. 6).

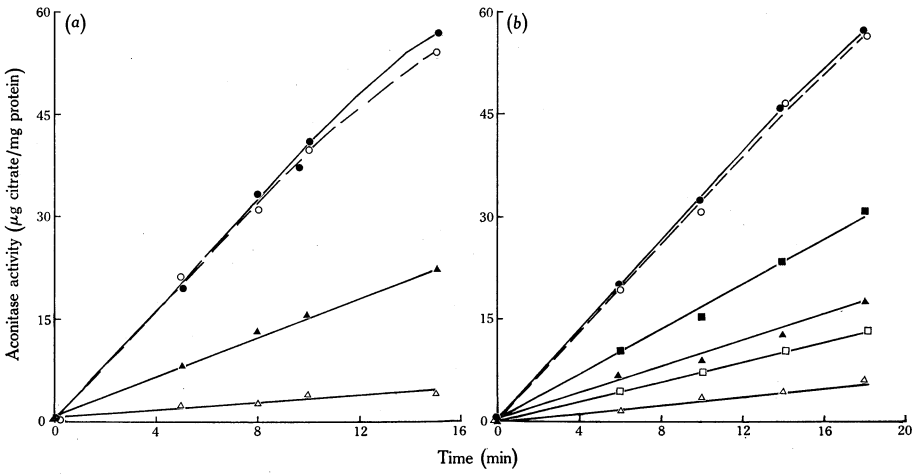

Fig. 5. Effect of GSH on fluorocitrate synthesis and aconitate hydratase inhibition in liver acetone powder preparations of (*a*) Western Australian and (*b*) South Australian possums. The complete incubation mixture was as described for Table 1 with the addition of GSH (10 mM). Tubes were incubated at 37°C for 45 min prior to addition of isocitrate. 1·0 ml 15% (w/v) TCA was added to individual tubes at the time intervals indicated and citrate was determined. ○ Without fluoroacetate. ● Without fluoroacetate; 10 mM GSH. □ 2·5 mM fluoroacetate. △ 5·0 mM fluoroacetate. ■ 2·5 mM fluoroacetate; 10 mM GSH. ▲ 5·0 mM fluoroacetate; 10 mM GSH.

The isolation of *S*-carboxymethylglutathione and *S*-carboxymethylcysteine from lettuce plants treated with fluoroacetate (Ward and Huskisson 1972) suggests that the defluorination mechanism is similar in plant and animal tissues but is distinct from the bacterial system.

Sternburg *et al.* (1953) have demonstrated that the DDT resistance of house flies can be related to increased levels of a DDT dehydrochlorinase, while Tahori (1963) has shown that fluoroacetate-resistant house flies are cross resistant to DDT.

It is tempting to postulate that the considerable differences in fluoroacetate tolerance between Western Australian and South Australian possums is related to different levels of a dehalogenating enzyme such as the glutathione-*S*-alkyl-transferase (EC 2.5.1.12) described here. No difference was found, however, in the defluorination rates *in vitro* between animals from the two populations (Table 2).

It is also apparent from the rapidity of conversion of fluoroacetate into fluorocitrate that the rate of defluorination is sufficient to provide immediate protection.

Provided the animal can be partially protected from the toxic effects of fluoroacetate by some other mechanism, defluorination could then eliminate a considerable quantity of the toxin in a matter of hours.

It has been suggested that differences in tolerance to fluoroacetate poisoning may be related to differing sensitivities of aconitate hydratase to fluorocitrate or to differing abilities to convert fluoroacetate to fluorocitrate (Peters 1972). There appears to be little evidence to support this.

Both Western and South Australian possums rapidly converted fluoroacetate to fluorocitrate (Table 1) and aconitate hydratase activity in acetone powder preparations from both animals was similarly and competitively inhibited by fluorocitrate (Fig. 5). If inhibition of aconitate hydratase by fluorocitrate is the mode of toxic action of

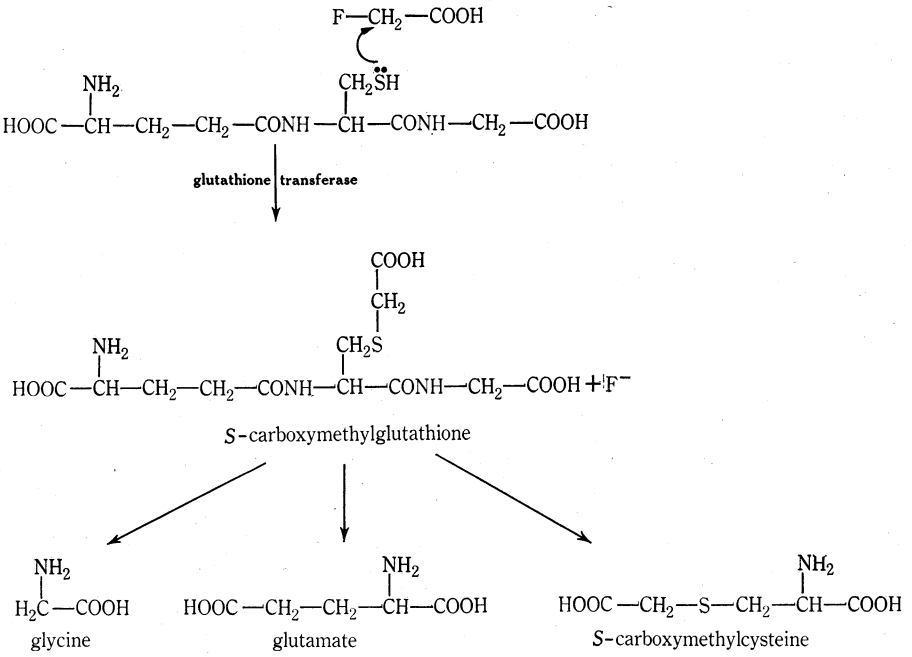

Fig. 6. Proposed mechanism of defluorination of fluoroacetate by possum liver.

fluoroacetate, it is difficult to understand the vast difference in citrate accumulation *in vitro* between animals from the two populations (Figs 2 and 3). Furthermore the competitive nature of the inhibition should ensure that the toxicity is diminished, as citrate accumulates. This, however, does not eventuate *in vivo*.

As the enzyme source used in this study was an acetone powder preparation in which intact membranes do not occur, the data suggest that *in vitro* differences in toxicity may be evident only in the presence of intact mitochondria. Very recently Kun *et al.* (1977) and Kirsten *et al.* (1978) have obtained convincing data to indicate that the principal mode of toxic action of fluorocitrate is a result of its inhibition of citrate transport through mitochondrial membranes rather than its inhibition of aconitate hydratase. They have found that fluorocitrate forms a thiol–ester bond with the sulfhydryl groups of two glutathione-dependent enzymes in the mitochondrial membrane. These enzymes normally catalyse both the formation of a citryl–thiol–ester

between citrate and oxidized glutathione (GSSG) and the subsequent cleavage of the ester bond, with the result that citrate is transferred through the membrane. The covalent binding of fluorocitrate to the SH groups of these two enzymes results in inhibition of citrate influx and efflux. Differences in citrate accumulation in the blood of Western Australian and South Australian possums (Figs 2 and 3) may therefore reflect differences in the inhibition of citrate entry into or exit from mitochondria rather than any difference in fluorocitrate formation, defluorination or aconitate hydratase inhibition.

E. Kun (unpublished data) has also suggested that GSH promotes the binding of fluorocitrate to these enzymes. The protective effect of GSH on aconitate hydratase inhibition as shown in this paper (Fig. 6) is compatible with this suggestion. In the absence of intact mitochondrial membranes the increased binding of fluorocitrate to protein SH groups would make less fluorocitrate available for aconitate hydratase inhibition. Our data can also be explained, however, by proposing the formation of a fluorocitryl–thiol ester between fluorocitrate and GSH itself.

It has also been shown that GSH at a concentration of 25 mM stimulates aconitate hydratase activity (Morrison 1954). The activation of aconitate hydratase by GSH appears to be related to the formation of a GSH-Fe^{2+} complex. GSH at a concentration of 10 mM, however, has little effect on aconitate hydratase activation (Morrison 1954), a result confirmed in this paper (Fig. 6), but such concentrations may inhibit the binding of fluorocitrate to the active site of the enzyme. Further studies are required to elucidate the mode of protective action of GSH and to distinguish between these possibilities.

Glutathione appears to play a central role in fluoroacetate metabolism and studies are currently underway to ascertain the importance of liver glutathione levels in the manifestation of fluoroacetate toxicity. The possibility that non-physiological sulfhydryl compounds may possess antidotal properties is also being investigated.

Acknowledgments

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References

Bettini, S., and Boccacci, M. (1958). R.C. 1st Supp. Sanit. **21**, 278. Cited by Cohen, A. J., and Smith, J. N. (1964). *Biochem. J.* **90**, 449–56.

Booth, J., Boyland, E., and Sims, P. (1961). An enzyme from rat liver catalysing conjugations with glutathione. *Biochem. J.* **79**, 516–24.

Boyland, E., and Chasseaud, L. F. (1969). The role of glutathione and glutathione *S*-transferases in mercapturic acid biosynthesis. *Adv. Enzymol. Relat. Areas Mol. Biol.* **32**, 173–9.

Boyland, E., and Williams, K. (1965). An enzyme catalysing the conjugation of epoxides with glutathione. *Biochem. J.* **94**, 190–7.

Camp, B. J., and Farmer, L. (1967). A rapid spectrophotometric method for the determination of citric acid in blood. *Clin. Chem. (Winston-Salem, N.C.)* **13**, 501–5.

Dabre, A., and Islam, A. (1968). Gas-liquid chromatography of trifluoroacetylated amino acid methyl esters. *Biochem. J.* **106**, 923–5.

Fjellstedt, T. A., Allen, R. H., Duncan, B. K., and Jakoby, W. B. (1973). Enzymatic conjugation of epoxides with glutathione. *J. Biol. Chem.* **248**, 3702–7.

Goldman, P. (1965). The enzymatic cleavage of the carbon–fluorine bond in fluoroacetate. *J. Biol. Chem.* **240**, 3434–8.

Horiuchi, N. (1960). Micro determination of fluorine in living organisms. VI. Stability of the C–F link in soil. *Takamine Kankyusho Nempo* **12**, 310–13.

Johnson, M. K. (1966). Studies on glutathione *S*-alkyltransferase of the rat. *Biochem. J.* **98**, 44–56.

Kelly, M. (1965). Isolation of bacteria able to metabolize fluoroacetate or fluoroacetamide. *Nature (London)* **208**, 809–10.

King, D. R., Oliver, A. J., and Mead, R. J. (1978). The adaptation of some Western Australian mammals to food plants containing fluoroacetate. *Aust. J. Zool.* **26**, 699–712.

Kirsten, E., Sharma, M. L., and Kun, E. (1978). Molecular toxicology of (−)erythro-fluorocitrate: selective inhibition of citrate transport in mitochondria and the binding of fluorocitrate in mitochondrial proteins. *Mol. Pharmacol.* **14**, 172–84.

Kun, E., Kirsten, E., and Sharma, M. L. (1977). Enzymatic formation of glutathione–citryl thioester by a mitochondrial system and its inhibition by (−)erythro-fluorocitrate. *Proc. Natl Acad. Sci. U.S.A.* **74**, 4942–6.

Morrison, J. F. (1954). The activation of aconitase by ferrous ions and reducing agents. *Biochem. J.* **58**, 685–92.

Morrison, J. F., and Peters, R. A. (1954). Biochemistry of fluoroacetate poisoning. The effect of fluorocitrate on purified aconitase. *Biochem. J.* **58**, 473–9.

Oliver, A. J., King, D. R., and Mead, R. J. (1977). The evolution of resistance to fluoroacetate intoxication in mammls. *Search* **8**(4), 130–2.

Pesce, M. A., and Strande, C. S. (1973). A new micro-method for determination of protein in cerebrospinal fluid and urine. *Clin. Chem.* **19**, 1265–7.

Peters, R. A. (1972). In 'Carbon–Fluorine Compounds'. A Ciba Foundation Symposium. pp. 56–8. (Associated Scientific Publishers: Amsterdam.)

Peters, R. A., Shorthouse, M., Ward, P. F. V., and McDowell, E. M. (1972). Observations upon the metabolism of fluorocitrate in rats. *Proc. R. Soc. London Ser. B* **182**, 1–8.

Preuss, P. W., and Weinstein, L. H. (1969). Studies on fluoro-organic compounds in plants. II. Defluorination of fluoroacetate. *Contrib. Boyce Thompson Inst.* **24**, 151-5.

Smith, F. A., Gardner, D. E., and Yuille, C. L. (1977). Defluorination of fluoroacetate in the rat. *Life Sci.* **20**, 1131–8.

Sternburg, J., Vinson, G. B., and Kearns, C. W. (1953). Enzymatic dehydrochlorination of DDT by resistant flies. *J. Econ. Entomol.* **46**, 513–15.

Tahori, A. S. (1963). Selection for a fluoroacetate resistant strain of house flies and investigation of its resistance pattern. *J. Econ. Entomol.* **56**, 67–9.

Ward, P. F. V., and Huskisson, N. S. (1972). The metabolism of fluoroacetate in lettuce. *Biochem. J.* **130**, 575–87.

Wilkinson, G. N. (1961). Statistical estimations in enzyme kinetics. *Biochem. J.* **80**, 324–33.

Yllner, S. (1971). Metabolism of chloroacetate-1-^{14}C in mouse. *Acta Pharmacol. Toxicol.* **30**, 69–80.

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