The Use of ^{13}C-Labelling to prove that Creatinine is oxidized by Mammals into Creatol and 5-Hydroxy-1-methylhydantoin

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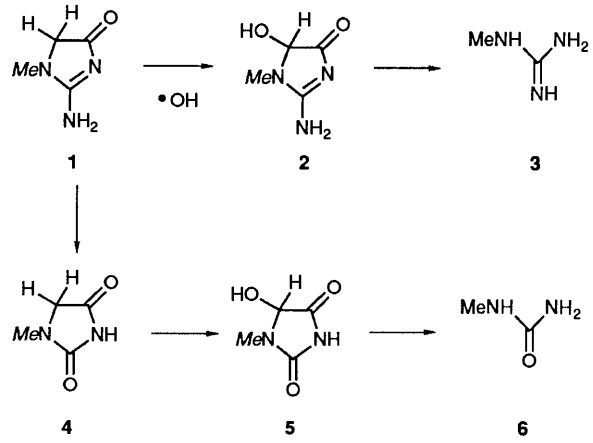
Creatinine **1** is not an end-metabolite but a precursor for three heterocycles, 2-amino-5-hydroxy-1-methyl-4(5*H*)-imidazolone (creatol) **2**, 1-methylimidazolidine-2,4-dione **4** and its 5-hydroxy derivative **5**; this is now proven directly by administration of [*Me*-^{13}C]creatinine **1**, to a uraemic rat and subsequent isolation of the corresponding labelled metabolites **2**, **4** and **5** from its urine.

Creatinine **1** can no longer be considered as an end-metabolite in mammals. Thus, we have isolated two methylhydantoins, 1-methylimidazolidine-2,4-dione **4** and its 5-hydroxy derivative **5**, initially from inflamed rabbit skin tissues[1] and subsequently from the urine of uraemic patients;[2] moreover, we have isolated creatol, 2-amino-5-hydroxy-1-methyl-4(5*H*)-imidazolone **2** from the same urine.[2] These facts led to the proposal of dual oxidative catabolic pathways for **1**, at least in uraemic patients (Scheme 1).[2–6] We have already shown that mammals can convert the two methylhydantoins **4** and **5** into methylurea **6** and the creatol **2** into methylguanidine **3**,[3,4,6] but the initial steps, **1** to **2** and **1** to **4**, have not been rigorously proven *in vivo*, mainly owing to the low conversion rates. We now offer such direct proof by ^{13}C-labelling of the substrate **1** and subsequent isolation of the labelled metabolites **2**, **4** and **5**.

[*Me*-^{13}C]Creatinine **1**, which contained more than 99% of *Me*-^{13}C (MSD Isotopes, Canada), was administered (i.p.; 500 mg kg^{-1}) into a uraemic rat (bodyweight : 200 g) with adenine-induced chronic renal failure:[7] urine (44 ml) was collected during the next 24 h. The three creatinine metabolites **2**, **4**, and **5** were isolated therefrom by methods previously used for their endogenous unlabelled analogues,[1–4] and the ^{13}C-content of each was estimated by ^{13}C and ^{1}H NMR spectroscopy. The higher serum creatinine (s-Cr) level of the rat (*ca.* 25 mg dm^{-3}) than that of normal rat (*ca.* 3–5 mg dm^{-3}) showed the rat suffered from renal failure.[7]

A part (1 ml) of the urine was stored for reference, and the remainder was applied to a PK 216 ion exchange column (H^+ form). After washing with water, the eluate with ammonia (2 mol dm^{-3}) was evaporated *in vacuo* to give a residue, which was dissolved in water and applied to a Biolex-70 ion exchange column (H^+ form). After washing with water, the eluate with acetic acid (0.1 mol dm^{-3}) was evaporated *in vacuo* to give **2**, (0.66 mg, 0.11%), determined by reversed-phase HPLC analysis as previously reported.[2] The water washings from the PK 216 column were neutralized and evaporated *in vacuo* to dryness. From the residue, the hydantoins **4** and **5** were separated on a silica gel column (ethyl acetate) in yields of 1.51 mg (0.30%) and 0.87 mg (0.15%), respectively. The amounts of **4** and **5** were determined by HPLC analysis on an ODS column as previously reported.[1–4]

Each of the isolated metabolites **2**, **4** and **5** was shown to be derived almost entirely from the administrated (extrinsic) [*Me*-^{13}C]creatinine **1** by its ^{13}C NMR off-resonance spectrum. Thus the labelled specimen **2** showed a single peak for its ^{13}C-Me group at δ 30, whereas an unlabelled specimen from natural (intrinsic) **2** showed three additional signals (Fig. 1); likewise, the isolated specimens of **4** and **5** showed only single peaks at δ 30 and 28, respectively. Even more precise information was obtained on the ratio of extrinsic to intrinsic material in each isolated metabolites by the ^{1}H NMR spectra: the protons of the ^{13}C-Me group appeared as a doublet whereas those of the (natural) ^{12}C-Me group appeared as a singlet. The spectrum of the specimen of **2** showed a large doublet (*J* 143 Hz) and a small singlet, both at δ 3.11, the integration of which showed that the specimen contained *ca.* 95% of extrinsic (labelled) material; the metabolites **4** and **5** showed similar peaks centred at δ 2.95 (*ca.* 90%, extrinsic) and (*ca.* 95%, extrinsic) 2.90, respectively.



Scheme 1 Dual oxidative creatinine catabolic pathways in mammals. *Me* shows the ^{13}C-labelled methyl group in the specimens, isolated from the urine after administration of [*Me*-^{13}C]creatinine.

Table 1 Urinary levels of **1** and its oxidative metabolites **2**, **3** and **5**

Rat	**1** mg day^{-1}	**2** μg day^{-1}	**3** μg day^{-1}	**5** μg day^{-1}
Normal ($b = 15$)[a]	8.00 ± 0.47	10.1 ± 0.6	8.6 ± 0.4	145.8 ± 14.7
Uraemic ($n = 6$)[b]	2.21 ± 0.19	30.1 ± 3.5	27.5 ± 3.7	32.0 ± 7.0
Human	g day^{-1}	mg day^{-1}	mg day^{-1}	mg day^{-1}
Healthy ($n = 10$)	1.48 ± 0.14	2.25 ± 0.21	0.39 ± 0.06	4.13 ± 0.42

[a] Serum creatinine (s-Cr) was 0.30 ± 0.13 mg dm^{-3}. [b] Uraemia was induced by adenine (s-Cr was 3.40 ± 0.13 mg dm^{-3}). All data are shown as mean ± standard error.

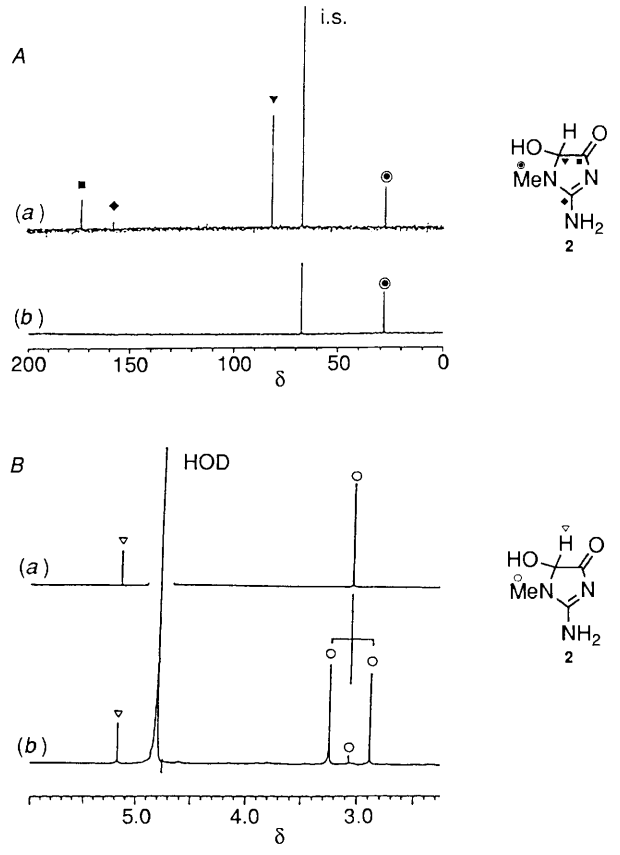


Fig. 1 ^{13}C and ^{1}H NMR spectra of intrinsic and isolated creatol **2**. *A* ^{13}C-NMR off-resonance spectra in D_2O [internal standard (i.s.): dioxane]. *B* ^{1}H NMR spectra in D_2O (i.s.: Bu^tOH). (*a*) Intrinsic CTL (2). (*b*) Isolated **2** from the urine after administration of [*Me*-^{13}C]-creatinine **1** in a uraemic rat.

These results show that **1** is indeed the precursor of the three heterocycles **2**, **4** and **5** and, in addition, that the metabolites **2**, **4** and **5** do exist naturally, albeit in small amounts, in the urine of uraemic rats. Thus, the oxidative catabolism of creatinine (Scheme 1) is now realistic in uraemic mammals. However, it remained to be shown whether the same metabolites occurred in normal (non-uraemic) mammals.

Accordingly, urine samples from human subjects and rats (both normal and uraemic) were analysed for **1**, **2**, **3** and **5** by HPLC methods previously outlined.[1-4] As indicated in Table 1, all urine samples contained all three metabolites (**2**, **3** and **5**) thus suggesting that both catabolic pathways for **1** in Scheme 1 are generally applicable. Although renal dysfunction clearly decreased the clearance of **1** and **5**, it also increased levels of **3** (a uraemic toxin)[8,9] and **2** (its precursor). This could be so if the production **2** and **3** is stimulated by active oxygen species and some preliminary results do indicate the hydroxyl radical as the most likely contributor.[2,5,10,11] In addition, **2** and **3** are both accumulated in the sera of uraemic animals (data not shown) so we are investigating whether their abnormal concentrations therein may offer diagnostic indexes for the degree of renal dysfunction.

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