# $^1$H Spin Echo NMR Evidence for the Interaction of Chromium(VI) with Glutathione in Intact Erythrocytes

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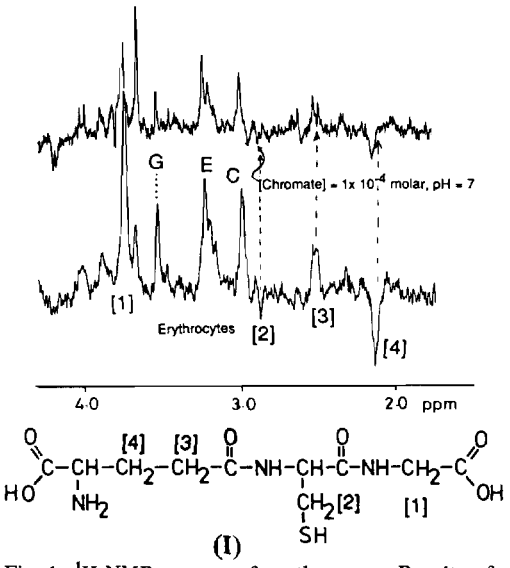
There is considerable current interest in the carcinogenicity and mutagenicity of chromium(VI) [1, 2]. In neutral aqueous solutions the dominant chromium(VI)-containing species is the chromate ion $[CrO_4]^{2-}$. Wetterhahn and Connet [1] have pointed out that this ion can readily cross cellular membranes, via non-specific anion carriers, and that reduced species generated within the cell are probably the active toxins *in vivo*; this has been termed the 'uptake–reduction' model. *In vitro* experiments on chick embryos suggest [3] that glutathione (γ-glutamylcysteinylglycine, GSH) may have a potentiating effect on the toxicity of chromate *in vivo*. We [4], and others [5], have shown that chromium(V) species can be generated from the reaction of GSH with chromate at neutral pH, under a wide range of conditions.

In this communication we wish to report our observation of evidence for the direct interaction of chromate with GSH in intact erythrocytes. This supports a hypothesis based on a reaction between chromium(VI) and GSH leading to a toxic intermediate responsible for the mutagenicity and carcinogenicity of the chromate ion.

## $^1$H NMR Studies of Intact Human Erythrocytes

In Fig. 1 are shown typical results of spin echo experiments on erythrocytes both before and after the addition of chromate to the suspension. The signals due to glutathione, creatine (C), glycine (G) and ergothioneine (E) are readily identified by reference to the comprehensive study carried out by Rabenstein *et al.* [6]. Assignments for glutathione (I) are indicated in the Figure.

In the spectrum before the addition of chromate, signals characteristic of glutathione (I) (1 to 4), creatine (C), ergothioneine (E) and glycine (G) are all clearly visible. In the spectrum after the addition of chromate, signals in positions close to those originally assigned as ergothioneine, creatine and glycine are still observed. However, at the concentration used for the above experiment ([chromate] = $1 \times 10^{-4}$ mol dm$^{-3}$ in saline $D_2O$) the glutathione spectrum is effectively obliterated. Similar results are obtained at other concentrations of chromate.




(I)

Fig. 1. $^1$H NMR spectra of erythrocytes. Results of a typical experiment: initial spectrum of erythrocytes, 5-min acquisition; and a 5-min acquisition 5 min after the addition of chromate ($[CrO_4^{2-}] = 1 \times 10^{-4}$ mol dm$^{-3}$; pH = 7).

In general we have observed that the signals from the $-CH_2$ groups adjacent to either carboxylates or the RSH function are particularly susceptible to the addition of chromate. The disappearance of the GSH NMR spectrum could be due to spin relaxation effects due to paramagnetic chromium(V) (or a chromium(III) species) or broadening due to rapid exchange. In view of the lability and paramagnetism of chromium(V) complexes and the increasing evidence for their formation [4] in such systems, we favour explanations involving a chromium(V) complex. Although any exact conclusion concerning the species generated is not possible from this work, we have shown a strong interaction between chromate and GSH to occur in an intact cellular system. The fact that a much weaker interaction, if any, is detected for the other cellular components supports a special role for glutathione in mediating chromate toxicity.

## Experimental

Erythrocytes were separated by centrifugation and washed two or three times with normal saline ($D_2O$). NMR spectra were recorded with a Bruker WH400 pulsed Fourier transform NMR spectrometer. The spin echo method, using a 0.060 s delay time as described by Rabenstein *et al.* [6], was used to

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collect the data. Spectra were recorded before the addition of chromate and at intervals after the addition of chromate, typically 5-min acquisitions at 5-min intervals. The results of one experiment typical of several performed are shown in Fig. 1.

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