# Chemical Models Important in Understanding the Ways in which Chromate Can Damage DNA

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Chromate is an established human carcinogen. There have been many studies of the reactivity of chromate aimed at improving understanding of chromate toxicity. In the present paper a number of conclusions of these studies are reviewed and considered in the light of new results obtained in our laboratories. A number of hypotheses are considered; it is concluded, however, that it is impossible to reconcile the generation of strand breaks by chromate during its reduction by glutathione with any simple mechanism involving the generation of DNA lesions by free hydroxyl radicals. Kinetic, spin-trapping, and competition kinetic studies, based on a strand-breaking assay, are reported in support of this conclusion. — Environ Health Perspect 102(Suppl 3):3–10 (1994).

Key words: chromate, chromium(VI), chromium(V), glutathione, ascorbic acid, electron spin-trapping, resonance spin-trapping, DNA strand breaks, competition kinetics

## Introduction

The toxicity of chromium(VI) has been extensively studied in the last few years (*1,2*). Interest can be traced to the fact that chromate is an established human carcinogen that is, because of its reactivity, particularly amenable to mechanistic studies (*3–6*). However, the complicated redox chemistry of chromium in aqueous solution has so far prevented a clear picture as to which mechanisms are important in the formation of DNA lesions both *in vitro* and *in vivo*. In terms of understanding the *in vivo* effects, one particularly important study has shown that chromate is carcinogenic in rats by inhalation; for some reason this important study, in which chromate was administered by the route most appropriate to model the suggested effects in humans, is frequently overlooked (*7*).

The majority of mechanistic studies have focused on the reduction of chromate by glutathione (*8–13*). However, ascorbate has recently been shown to be the stoichiometrically most important reductant in some biological fluids and cellular systems (*14–16*). Many workers have sought to establish a crucial role for the hydroxyl radical (*9–11,17,18*) and/or Fenton or pseudo-Fenton chemistry and have reported that hypervalent chromium complexes, in the presence of added (mM) hydrogen peroxide, can generate DNA lesions and spin adducts usually taken as characteristic of hydroxyl radicals (11–13). However, the concentration of hydrogen peroxide in biological systems is controlled at a very low (<1≤M) level, by protective enzyme systems, and model systems involving added peroxide are unlikely to be particularly relevant to the *in vivo* situation.

Even so, a large number of papers have addressed the problem of the generation of hydroxyl radicals and related species during the reduction of chromate. Kawanishi and co-workers studied the reaction of hydrogen peroxide with chromate at neutral pH (*19*) and were able to present convincing evidence for hydroxyl radical generation using the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) both in the presence and absence of ethanol. Some evidence for a peroxy-chromium(V) species was also presented. In related work Shi and Dalal have used the well-known compound potassium tetraperoxychromate(V) to generate small quantities of hydroxyl radical species (*18*); larger quantities of radical species were generated by chromium(V) species, such as an NADPH complex, and in the presence of added $H_2O_2$ (*20*). Wetterhahn and co-workers have shown using DMPO (alone) that the hydroxylated spin trap derived from DMPO can be generated in large amounts by adding hydrogen peroxide to a preincubated solution of chromium(VI) and glutathione, i.e., a solution containing significant amounts of partially reduced chromium species (especially Cr(V) and potentially Cr(IV)) (*10,11*).

It was consequently proposed that oxidative damage caused by chromate may be mediated *in vivo* by a related mechanism in which chromium intermediates catalyze the formation of reactive oxygen species via peroxy Cr(V) complexes. Some workers have even suggested the direct involvement of $[CrO_8]^{3-}$ ions *in vivo*. The last suggestion seems particularly unlikely as the tetraperoxy species is only stable in alkali solution in the presence of a large excess hydrogen peroxide (*21*), as may be appreciated from the following equation:

$$2[CrO_4]^{2-} + 9H_2O_2 + 2OH^- \rightarrow 2[Cr(O_2)_4]^{3-} + 10H_2O$$

Although such mechanisms may incorporate some features potentially important in understanding chromium toxicity, several important observations concerning DNA *in vitro* are not explained by a pseudo-Fenton or peroxychromate mechanism. Farrell et al. (*22*) have shown that the relatively stable Cr(V) complex sodium bis[2-ethyl-2-hydroxybutanoato(2-)oxochromate(V)] ($[Cr(ehba)_2O]^-$) (*23*) can cause

This paper was presented at the Second International Meeting on Molecular Mechanisms of Metal Toxicity and Carcinogenicity held 10–17 January 1993 in Madonna di Campiglio, Italy.

We are grateful for the support of work on chromate toxicity at Q.M.W. by the Cancer Research Campaign, and the European Science Foundation (for a fellowship to A.K.).

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strand breaks in supercoiled DNA, and in two papers Kortenkamp et al. have discussed the formation of strand breaks by chromate/glutathione, or a chromium(V) containing material derived from GSH and chromate (*8,9*). None of the above studies involved the use of added hydrogen peroxide. Wetterhahn et al. had never observed strand breaks in the absence of added hydrogen peroxide. However, quite recently, in a study aimed principally at understanding the effect of chromium binding on the activity of restriction endonucleases such lesions were observed in pBR322 DNA (dialyzed to remove metal ions) (*24*).

Consequently, a number of important aspects of the formation of DNA lesions, characteristic of oxidative damage, are far from clear. The present article aims to draw attention to the full range of reaction pathways which might link high oxidation state chromium chemistry and oxidative damage to DNA. The results of spin-trapping and competition-kinetic experiments designed to distinguish between individual mechanisms relevant to chromate genotoxicity are reported. The present work concentrates on the mechanisms by which chromate might cause oxidative damage, especially strand breaks, on reduction by biologically important reducing agents.

## Mechanisms by Which Chromate May Cause Oxidative Damage

The mechanisms by which chromium might cause oxidative damage to DNA include:

1. Fenton-type chemistry, which can be conveniently classified as: *a)* pseudo-Fenton chemistry involving high oxidation state chromium complexes (*8,17*) such as:

$$Cr^{(n-1)+} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + Cr^{n+}$$

involving oxidation states (V) or (IV). In the presence of thiols chromium may redox cycle by:

$$Cr^{n+} + RS^- \rightarrow Cr^{(n-1)+} + RS^{\bullet}$$

There are several papers which suggest that pseudo-Fenton chemistry involving chromium might be important and some of these invoke the direct involvement of the species $[Cr(O_2)_4]^{3-}$. *b)* Fenton chemistry involving hydrogen peroxide generation in a chromium mediated step, i.e., classical ·OH generation by iron (*9–13*):

$$Fe^{2+} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

which to be chromium mediated would follow a chromium-initiated step which might include (*8,17,25,26*):

(i)

$$Cr^{VI} + RSH \rightarrow Cr^{VI}SR$$
$$Cr^{VI}SR \rightarrow Cr^{V} + RS^{\bullet}$$
$$RS^- + RS^{\bullet} \rightarrow RSSR^-$$
$$RSSR^- + O_2 \rightarrow RSSR + {}^{\bullet}O_2^-$$
$$2{}^{\bullet}O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The potential of thiolates to generate superoxide is well documented (*25,26*), and under anaerobic conditions thiyl radicals can perform H-abstraction reactions (*27*). This pathway is unlikely to be important under aerobic conditions because the reaction of thiolates with molecular oxygen is very fast. There are chromium-dependent routes to superoxide, one being further mediated by the thiol, the other mediated by a high-oxidation state path:

(ii)

$$Cr^{n+} + O_2 \rightarrow Cr^{(n+1)+} + {}^{\bullet}O_2^-$$

The former has been described in a number of related systems. The feasibility of the latter route has not yet been considered. Both routes could provide the superoxide ion as required for Haber-Weiss/Fenton chemistry. The stability of a number of Cr(V) and (IV) complexes containing peroxy ($O_2^{2-}$) groups (*28,29*) suggests that electron transfer reactions between these entities are slow, which may mean that the redox potentials of oxygen and hypervalent species are quite similar.

2. The direct reaction of a highly oxidizing chromium species with DNA resulting in an oxygen transfer reaction (*22*) which may be generalized as:

$$[L_nCr^{V}{=}O]^{n+} + H\text{-}R \rightarrow [L_nCr^{III}]^{+} + ROH$$

3. More complicated radical pathways in which other radical species are generated, for example, for amino acids there is evidence that $RNH_2^{+\bullet}$ species can be generated by hypervalent (high-oxidation state) iron complexes (*30*).

4. The generation of chromium in intermediate oxidation states by enzyme systems.

High-oxidation state iron chemistry provides some useful analogies. Cytochrome $P_{450}$, and related oxidizing systems generate ferryl species (formally Fe(V)=O), which are involved in direct oxygen transfer reactions. Related reactions include the epoxidation of alkenes by metallo-oxo species (*31*). In contrast, many oxidations effected by iron and peroxides are mediated by ·OH dependent Fenton-type chemistry (see above). However, many porphyrin-based systems intended as enzyme models in fact operate via alkoxy radical chemistry. Barton and Doller developed a number of iron-based oxidizing systems which have been suggested to function via oxygen-transfer reactions (*32*). However, recent studies of such systems may suggest that radical processes are important in these oxidizing systems (*33*).

## Materials and Methods

Sodium perchlorate and sodium dichromate were from BDH Chemicals Ltd. (Poole, UK) [4-(2-hydroxyethyl)]-1-piperazine-ethanesulfonic acid (HEPES), dimethyl sulfoxide (DMSO), ethanol, and methanol were from the Aldrich Chemical Co Ltd. (Gillingham,UK). Glutathione and 5,5-dimethyl-1-pyroline-1-oxide (DMPO) were purchased from Sigma Biochemicals (Poole, UK). Sodium 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) was synthesized by the method of Kaur et al. (*34*) or purchased from Sigma. The commercial material has appeared to be contaminated with acid on some occasions, which has led to some problems with buffering. Stabilizer-free hydrogen peroxide was supplied by Interox Ltd. (Warrington, UK). Distilled water was further purified by demetallation with chelex resin (Biorad, Hemel Hampstead, UK) for the EPR and strand-break studies. The Cr(V) intermediate $Na_4Cr(GSH)_4.8H_2O$ was prepared as previously reported (*35,36*). Chromium(V) complexes of hydroxybutanoates were prepared by literature methods (*23*).

### Kinetics

The pH of solutions was measured by an EIL 7000 selective ion meter (Luton UK). Electronic spectra were measured using a Perkin-Elmer 330 spectrophotometer (Perkin-Elmer, Umberlingen, Germany). The absorbance of the chromate ion at 370 nm was followed. Freshly prepared stock solutions of the ligands, chromate, and buffer or distilled water were kept in the thermostatted water bath. The cell was thermostatted to the appropriate reaction temperature, and the required volume of the thermostatted stock solution of the ligands and buffer or distilled water was pipetted into a 1-cm spectrophotometer quartz cell. A small amount of the thermostatted chromate stock solution was injected into the reaction cell with a micro-

liter syringe. The cell was then stoppered, shaken, and returned to the thermostatted sample compartment of a Perkin-Elmer 330 spectrophotometer for continuous measurements of absorbance against time.

### Spin Trapping

Solutions of DBNBS ($2.5 \times 10^{-2}$ M) in phosphate buffer (0.1 M, pH 7.5) with 50% MeOH or 50% DMSO were prepared. To 2-ml aliquots of such solutions a typical procedure was to add 1 to 5 mg of the solid Cr(V) glutathione complex (*35,36*). The samples were shaken and immediately transferred into EPR tubes. Unless otherwise stated, the EPR spectra were recorded within 1 to 2 min of preparing the sample. For the experiments performed with the exclusion of oxygen, the EPR sample tubes and all the solutions were purged with nitrogen for at least 30 min before preparation of the sample mixtures. EPR spectra were recorded on Varian E-4 or Bruker ER/200/D instruments (Karlsruhe, Germany). Experiments involving DMPO (*37*) were carried out in a similar manner. Details of concentrations are given with the experimental results.

### Competition Kinetic Studies

The procedures used for studying strand breaks in supercoiled DNA are similar to those in our earlier studies and are detailed by Kortenkamp and O'Brien (this issue). Two experimental details are particularly important: the demetallation of the buffer (chelex resin) and DNA (dialysis against DTPA).

## Results and Discussion

### Mechanisms of Reductions

There have been a number of studies of the reduction of chromate by GSH and related thiols such as L-cysteine (*38–42*). At neutral pH, for GSH, the predominance of a two-electron step in which the glutathione thiolate ester, formed in a rapid preequilibrium step (*38–40*), is reduced by a second mole of GSH is generally accepted. Figure 1 summarizes some features of the reaction scheme normally considered. The overall rate law in the presence of excess GSH becomes:

$$k_{obs} = k_1 K[\mathrm{GSH}]^2/(1+K[\mathrm{GSH}])fl^2 \quad [1]$$

However, a one-electron, proton-mediated ($[H^+]^2$) pathway is observed for the reduction of chromate by GSH in acidic (*38*) solution. This pathway would be very slow at neutral pH. However, it remains hard to account for the observation of the thiyl radical, as frequently seen in spin-trapping studies, solely in terms of an initial two-electron reduction. If the Cr(V) species generated by disproportionation, undergoes a clean two-electron reduction, toxic, reactive intermediates are not likely to be generated. Consequently, we decided to investigate the possibility that a minor, but significant, one-electron pathway might operate at neutral pH and physiological concentrations of GSH.

We reasoned (*42*) that experimentally this hypothesis might best be investigated at low concentrations of the thiol for which the rate of reaction was more likely to be influenced by any one-electron route. In a series of experiments the loss of chromate was followed for 1 to 2 hr and the initial rate determined (approximately 1 to 2.5% of reaction, [GSH] = $5\text{-}15 \times 10^{-5}$ mol/l and $[CrO_4]^2 = 1 \times 10^{-4}$ mol $dm^{-3}$, HEPES 0.05 mole $dm^{-3}$, 25 C, pH 7.0 ). The results clearly show (Figure 2), that under these conditions the disappearance of chromate is first order in glutathione (rate = 0.025[GSH]) (Figure 2, curve B ). The expected rate law (*1*), predicted for a two- electron route, collapses to a quadratic at low concentrations of GSH.

In the absence of a pseudo-first-order excess of either reactant, it is hard to establish formally the order of the reaction. However, if we assume a first-order process, following an initial preequilibrium step ($K = 131$) (*26,35*), the rate constant for the disappearance of the thiolate ester would be $1.9 \times 10^{-4} s^{-1}$, making an approximate overall rate law for the disappearance of chromate under these conditions:

$$k_{obs} = \frac{(0.025\,[\mathrm{GSH}] + 0.358 \times 131[\mathrm{GSH}])}{(1+131[\mathrm{GSH}])} \quad [2]$$

Figure 2 compares the extrapolation of Equation 1 (curve A) with the experimentally observed results (curve B).

These results indicate that the one electron path for the reduction of chromate by GSH may be significant at physiological concentrations of GSH, e.g., at 5 mM GSH using Equation 2 we would predict the reaction to proceed initially by 10% one-electron reduction and 90% two-electron reduction. One-electron reduction could result in the release of thiyl radicals into the solution; these species represent one possible route to reactive oxygen species and strand breaks. Chromium(V) thus generated is likely to be free of ligands and more reactive than the relatively stable Cr(V) GSH complexes that have been identified in such solutions. We conclude that a one-electron route may be a significant source of reactive intermediates in the reduction of chromate by GSH at neutral pH.

The situation may well be similar for ascorbic acid, as radical intermediates are readily observed in the reaction of chromate and ascorbic acid (*43,44*) even though the stoichiometric mechanism may involve an initial clean two-electron reaction (*39*). The situation is further complicated for ascorbic acid by the stability of the ascorbic acid radical anion (*45,46*).

### Spin Trapping

In an attempt to assess the feasibility of various routes to oxidative damage, we have performed spin-trapping experiments

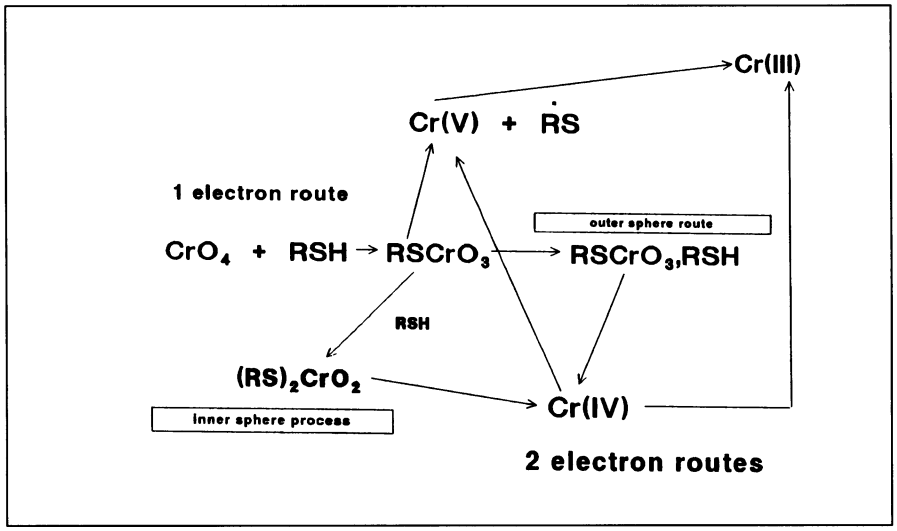


**Figure 1.** Reaction scheme showing the major suggested routes for the reduction of chromate by glutathione.

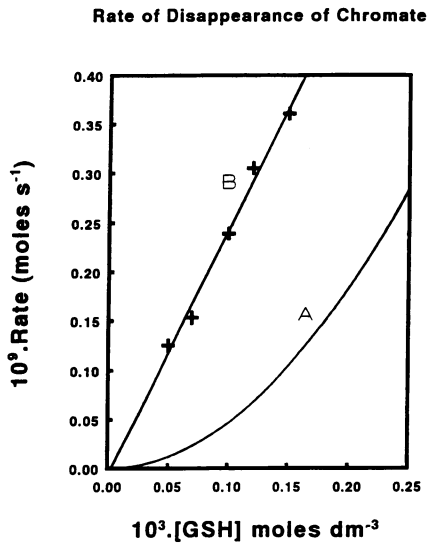


**Figure 2.** A comparison of the expected rate of initial chromate disappearance (curve A), as expected from IARC (*1*), with that experimentally observed at low chromate and GSH concentrations (curve B) (pH 7.0, 25°C, $[CrO_4]^{2-}$ = 1 x $10^{-5}$ mole $dm^{-3}$).

using a range of Cr(V)-containing species and Cr(VI) in the presence of various reductants. The water-soluble spin trap DBNBS (3,5-dibromo-4-nitrosobenzene sulfonate) in DMSO/aqueous solutions (50% DMSO) has been used. DBNBS does not yield spin adducts with hydroxyl radicals or other oxygen-centered species (*47–49*); but it does give intense spectra with methyl radicals, which may be generated, for example, via hydroxyl radical attacks on DMSO. The use of this trapping procedure can overcome some of the problems of identifying species other than thiyl radicals which have been encountered in earlier studies using the spin trap DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide). Some of the significant pathways operating in trapping studies are summarized in Figure 3.

The Cr(V) intermediate $Na_4Cr(GSH)_2GSSG).8H_2O$ (prepared as previously reported (*35,36*), at concentrations of 0.5 to 2 × $10^{-3}$ mole $dm^{-3}$ in aqueous, air-saturated solutions of DMSO (50%, vol:vol), produced the EPR spectrum characteristic of the DBNBS methyl radical adduct (Figure 4). Molecular oxygen appears to be essential for the formation of the methyl radical spin adduct, since the typical signals of the adduct failed to appear under anaerobic conditions (Figure 4). This observation indicates that, under these conditions, neither Cr(V) species nor thiyl radicals alone are able to produce DBNBS–methyl radical adducts. We have found that high concentrations of DMSO (>ca 30%, v/v) are needed to successfully trap $\cdot CH_3$ in this system, and that the addition of large quantities of glucose (>1 M) hardly affects the yield of the $\cdot CH_3$ adduct of DBNBS. At higher concentrations (>3 × $10^{-3}$ mole $dm^{-3}$) of the Cr(V) complex, another spin adduct, probably the nonspecific one-electron oxidation product of DBNBS (*47–49*), was observed in addition to the methyl radical adduct; this type of spectrum is shown in Figure 5.

In some earlier spin-trapping experiments using DMPO we suggested that the chromyl chloride anion on dissolution produced considerable quantities of the hydroxyl radical adduct (*50*). However, this species is probably generated by direct oxidation of the trap as the characteristic ethyl and methyl adducts are not observed when trapping is carried out in the presence of ethanol or methanol. The results obtained for $CrOCl_4$ with the DMSO/DBNBS system are similar to those with the Cr(V) GSH complex in that both the DBNBS methyl radical adduct and direct oxidation product can be observed. Interestingly, the exclusion of oxygen in these experiments was accompanied by an increase in intensity of the signals typical of nonspecific species and a decrease in the signal of the methyl radical adduct (Figure 5). These results again indicate that the presence of oxygen is important in the formation of methyl radicals in this system.

Chromium(V) complexes of hydroxy butanoates of the kind first prepared by Rocek (*23,51*) are known to be able to cause strand breaks in supercoiled DNA (*22*). The reaction of these compounds with spin-trapping systems is really quite sluggish and it was quite difficult to obtain spectra. However, millimolar concentrations of such compounds in the DBNBS/DMSO system led only to the generation of the nonspecific product of DBNBS described above.

We were interested to see if we could reproduce a recent result reported by Lefebvre and Pezerat (*44*). They observed that chromate (10 mM) when reduced by ascorbate (10 mM) in aerated buffered (phosphate, pH 7.0) solution in the presence of DMPO and formate, produced a strong signal characteristic of the formyl radical. We have now independently confirmed this observation, but in demetallated buffer (Figure 6). At similar concentrations but using GSH or ascorbic acid in the DBNBS/DMSO system, the $\cdot CH_3$ radical adduct is observed. These conditions are not close to physiological, but the 1:1 ratio of chromate and reductant may be forcing the generation of higher concentrations of reactive intermediates. Our results on the generation of strand breaks by ascorbic acid and chromate (reference) support this suggestion.

We conclude, from these spin-trapping studies, that a wide range of oxidizing species are generated by chromate during its reduction by GSH or ascorbate and by isolated Cr(V) complexes. In terms of understanding the toxicity of chromate, we can draw a number of conclusions: *a*) Molecular oxygen may be important; it certainly affects the outcome of spin-trapping experiments (Figures 4–6). *b*) There is a definite propensity for an as yet unidenti-

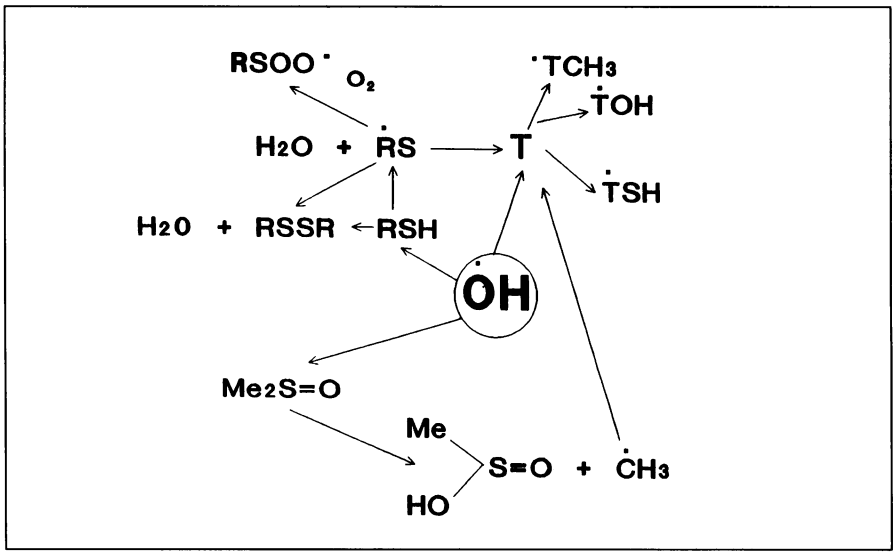


**Figure 3.** Trapping regimes showing the major species which might be trapped and how these might most simply be derived. T Spin trap.

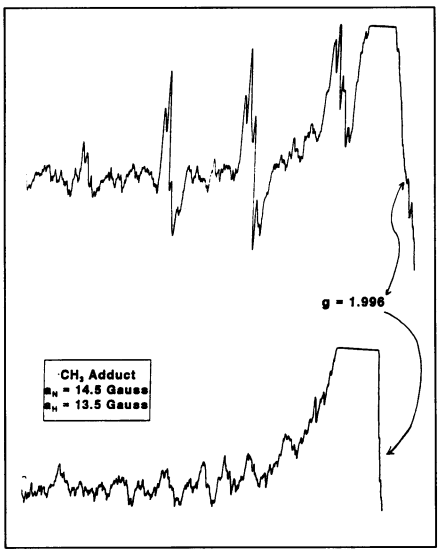


**Figure 4.** ESR spectra of nitrogen saturated solution of $Na_4Cr(GSH)_4.8H_2O$ (1.6 x $10^{-3}$ mole $dm^{-3}$) in phosphate buffer with 50% DMSO and DBNBS (0.1 mole $dm^{-3}$), lower trace; compared to an aerated solution, upper trace.

fied, but highly oxidizing, species to be generated. In several spin trapping systems the results are not fully consistent with the generation of free ·OH. Notably, $[CrOCl_4]^-$ does not produce the ethoxy radical adduct of DMPO in the presence of added ethanol. Similarly, in the ascorbate/Cr(VI) system only small quantities of the ·OH adduct are generated but large quantities of the formyl radical adduct are formed. Finally, in the DMSO/DBNBS system glucose is scarcely able to inhibit the formation of the $\cdot CH_3$ adduct.

It may be important that many of the species able to generate species characteristic of oxidation are also capable of coordination to metal ions, e.g., formate or DMSO; that might suggest a mechanism involving direct coordination of the secondary trap to the metal. Molecular oxygen also appears to be important in determining the products of these reactions. One mechanism that would reconcile some of these observations would be the formation of a high valency oxo- or molecular oxygen species of chromium capable of an oxygen transfer reaction.

What is clear is that a mechanism based solely on hydroxyl radical cannot explain all our spin-trapping results. We will now show that the strand breaks generated by chromate and glutathione cannot simply be explained in terms of a free hydroxyl radical mechanism.

## Strand Breaks in PM2 DNA

In order to assess if the hydroxyl radical can be held to cause the strand breaks caused by chromate and glutathione in the absence of added hydrogen peroxide, we have studied the effect of the radical scavenger DMSO on the induction of strand breaks by chromate and GSH. A concentration-dependent depression of DNA degradation is observed in the presence of DMSO; but glucose and sucrose, both strong hydroxyl radical scavengers, showed no protective influence on the induction of strand breaks at concentrations of up to 10 mM.

Competition kinetics can be used as a tool to quantify these observations and assess if the results are consistent with hydroxyl radical attack. It can easily be shown that:

$$N = N_0 k_{DNA}[DNA]/(k_s[S] + k_{DNA}[DNA]) \quad [3]$$

where

$N_0$ is the number of strand breaks in the absence of scavenger,

$N$ is the number of strand breaks in the presence of scavenger,

[S] is the concentration of scavenger,

[DNA] is the concentration of DNA-P

$k_s$ and $k_{DNA}$ are the bimolecular rate constants for the reactions of hydroxyl radical with scavenger and DNA, respectively.

Plots of the number of strand breaks against the concentration of scavengers should give a rectangular hyperbole with the limit $N_0$. For the reaction of DMSO with hydroxyl radical, $k_s$ ($7.0 \times 10^9$) is known (*52*). Using this value we predict that 50% inhibition of strand breaks would occur at 2.54 μM DMSO. However in the present system we find that no reduction in the number of strand breaks is observed until we reach millimolar concentrations of DMSO (Figure 7). As a control we have studied the inhibition of strand breaks as generated by hydrogen peroxide and iron(II); in this case only micromolar concentrations of DMSO are required. The calculated value of $15 \times 10^9 sec^{-1}$ $mole^{-1}l$ for the rate of ·OH with DNA, for the iron system, is still slightly higher that the literature value ($0.8 \times 10^9 sec^{-1} mole^{-1}$ l) There are two explanations for this observation: first, that PM2 DNA is a small and conformationally compact DNA probably much better able to compete for ·OH than ordinary double-stranded DNA; second that there may be a proximity effect, e.g., the coordination of Fe(II) to the DNA leading to an enhanced value for the apparent value of $k_{DNA}$.

## Conclusions

In terms of the mechanisms outlined in the introduction it is clear that a pseudo-Fenton mechanism can explain the results obtained *in vitro* in the presence of added $H_2O_2$. What is equally clear is that the mechanisms by which strand breaks are formed by chromate during its reduction in the absence of added hydrogen peroxide are not known, although there are some indications as to the most likely pathways. The significance of these pathways *in vivo* is unclear; but strand breaks are observed in exposed animals (*53*) as well as cells in culture (*54*). We are, however, in a position to make a preliminary comment on the feasibility of some of the other mechanisms.

We have previously speculated that the effects of GSH might be due to RS· radicals generating superoxide (*8,17*), leading to Fenton or pseudo-Fenton chemistry.

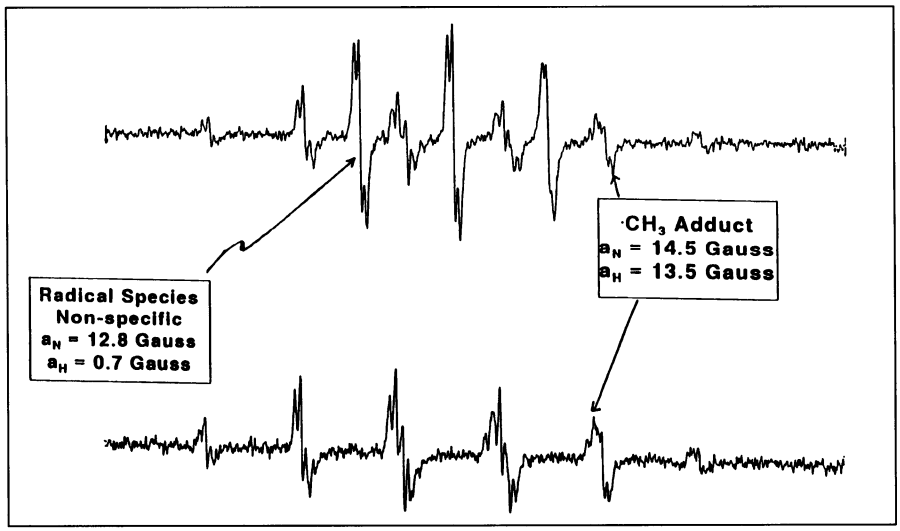


**Figure 5.** ESR spectra generated on the dissolution of $NEt_4[CrOCl_4]$ (1 mM) in a nitrogen-saturated solution, upper trace, and aerated solution, lower trace, of DBNBS (0.125 mole $dm^{-3}$), 50% DMSO (v:v) in phosphate buffer.

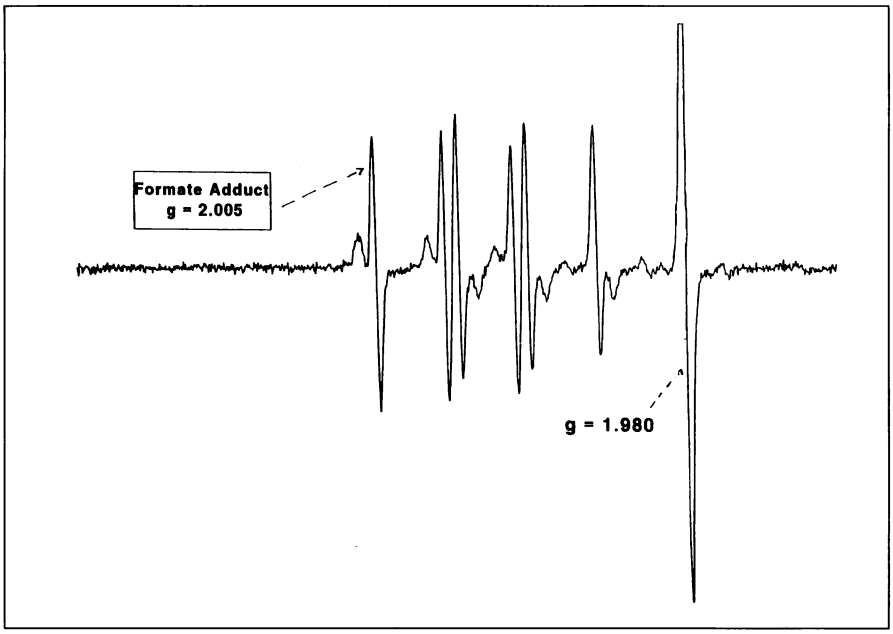


**Figure 6.** ESR generated by chromate (10 mM) and ascorbic acid (10 mM) in phosphate buffer, pH 7.0, approximately 10 min after mixing, in aerated solutions, in the presence of DMPO (50 mM) and formate (1 M). The buffer was carefully demetallated, and before the addition of chromate, ascorbic acid was stable in these solutions.

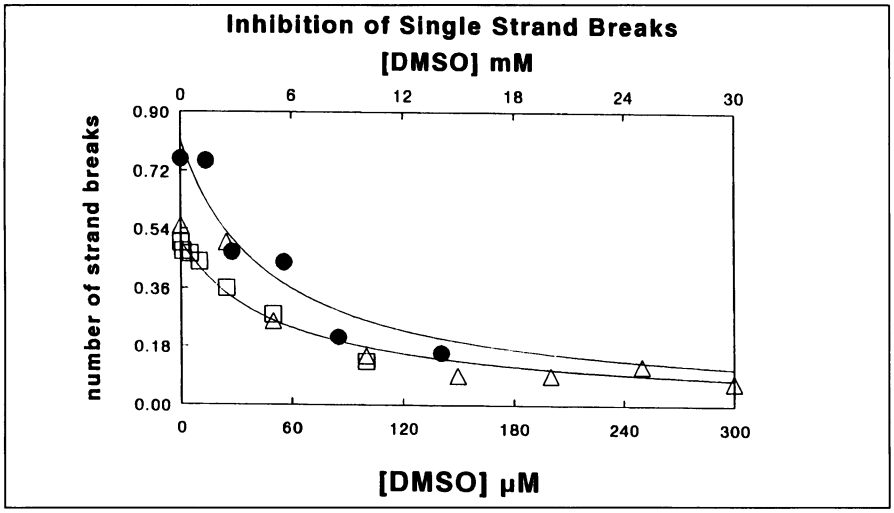


**Figure 7.** The inhibition of strand breaks in PM2 DNA (22 ≤M [DNA-P]) during the reduction of chromate (0.2 mM) by glutathione system ( 5mM) and as generated by iron(II) (50 ≤M) and hydrogen peroxide (400 ≤M).

We now believe this to be unlikely for the following reasons: *a*) competition kinetic studies are not consistent with the ultimate species leading to strand breaks being •OH; *b*) oxygen dependence is observed in spin-trapping studies with Cr(V) complexes even when these compounds do not contain thiolates; *c*). Farrel et al. (*22*) have shown that strand breaks can be formed by Cr(V) complexes which do not have thiolate functions; *d*) preliminary qualitative experiments in our laboratories suggest that superoxide and/or catalase have little or no effect on the yield of spin adducts from the Cr(V) GSH complex in the DBNBS/DMSO system. Mechanisms invoking the involvement of $[Cr(O_2)_4]^{3-}$ can be rejected as this species is not stable at neutral pH. A self-consistent picture for the mechanism by which chromate on reduction by GSH leads to strand breaks is yet to emerge. Our spin-trapping experiments suggest that both molecular oxygen and hypervalent chromium species are often required for the generation of radicals which may be important in mediating DNA lesions. The potential importance of Cr(IV) should not be overlooked (*55*).

The ability of ascorbate in combination with chromate to generate strand breaks is dealt with in other articles in this issue. However, the ascorbic acid system is, like that with GSH, complex, and detailed studies of this chemistry are only just starting.

In summary, all our results tend to support the hypothesis that DNA impairment and mutagenicity in these systems is mediated by the cytoplasmic reduction of chromate, and are in line with the suggestion that the outcome of the intracellular reduction is a toxifying effect. It is hard to reconcile the variety of damage caused by the *in vivo* reduction of Cr(VI) with a simple view of reduction as detoxification. Exogenous Cr(VI) may provide a challenge to mammalian cells to which they have not evolved a proper defense; and the wide range of effects observed may reflect relatively indiscriminate damage caused by chromate. We conclude that many mechanisms will be needed to fully understand the formation of oxidative lesions of DNA by chromate. Further studies of these systems are in progress in which we will continue to use well-defined Cr complexes. The article by Kortenkamp and O'Brien (this issue) reports our initial observations on the generation of strand breaks by chromate and ascorbate.

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