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VANADYL CAUSES HYDROXYL RADICAL MEDIATED DEGRADATION OF DEOXYRIBOSE

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Vanadyl caused a time- and dose-dependent degradation of deoxyribose to carbonyl products detectable with thiobarbituric acid. This process was inhibited by catalase, ethanol or HEPES; whereas superoxide dismutase was without effect. Vanadate did not substitute for vanadyl even in the presence of a source of O_2^- plus H_2O_2; but it did so in the presence of reductants such as thiols or NADH. It appears that hydrogen peroxide, generated by the autoxidation of vanadyl, is reduced by vanadyl to the hydroxyl radical; which, in turn, was responsible for the degradation of deoxyribose. A similar process might contribute to the toxic and pharmacological effects of vanadium salts.

KEY WORDS: Vanadyl, vanadate, hydroxyl radicals, deoxyribose.

INTRODUCTION

The effects of vanadium salts in biological systems have been extensively studied[1-3] and some of these effects are mediated by oxygen-derived free radicals.[2] Nevertheless the data concerning the production of HO· by vanadate/vanadyl are scanty and apparently contradictory.[4-13] Keller *et al.*[4] have suggested a key role for HO· in the $V_{(V)}$-dependent* oxidation of NAD(P)H by O_2^-, whereas we have shown that this process is independent of HO·. The same authors[4] used spin-trapping to detect a $V_{(V)}$-dependent production of HO· by the xanthine plus xanthine oxidase reaction; whereas others have obtained evidence for the vanadate-independent generation of HO· in this reaction mixture.[14-17] The ability of $V_{(V)}$ to mediate HO· production thus requires clarification.

We now describe the ability of $V_{(IV)}$ to generate HO·, with or without exogenous H_2O_2. We have also explored the possbility that $V_{(V)}$ could generate HO· during interaction with H_2O_2 and/or O_2^-.

MATERIALS AND METHODS

Ammonium metavanadate and uric acid were from Aldrich; 2-deoxyribose from Fluka; vanadylsulfate, xanthine oxidase (1 U/mg), catalase, and NADH, from Boehringer; xanthine and dithiothreitol from Koch-Light; and 2-thiobarbituric acid and HEPES from Merck. Glass-distilled water was used throughout and solutions of

*Abbreviations used: $V_{(V)}$ – vanadate; $V_{(IV)}$ – vanadyl; TBA – thiobarbituric acid; TBARS – TBS reactive substances; DR – 2 deoxyribose; TCA – trichloroacetic acid.

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vanadylsulfate, ammonium metavanadate, NADH, dithiothreitol and cysteine were freshly prepared, as needed.

Deoxyribose (DR) degradations was measured in terms of the formation of TBA-reactive substances (TBARS).[17] Reaction mixtures contained 3.5 mM DR, 25 mM sodium phosphate and the indicated concentrations of other components at pH 7.4 in a total volume of 1.0 ml. Reactions were carried out with shaking for 30 min at 37°C and were terminated by addition of 1.0 ml of 2.8 % TCA. One ml of TBA (1 % w/v in 50 mM NaOH) was then added and the samples were heated to 100°C for 15 min. After cooling A_{532} nm was measured. Xanthine oxidase reactions were performed with 0.3 mM xanthine and 5 μg/ml of xanthine oxidase in 25 mM sodium phosphate $\pm$ 25 μM cytochrome *c*. Urate production was followed at 295 nm cytochrome *c* reduction at 550 nm.

RESULTS

Vanadyl (line a), but not vanadate (line b), converted DR to TBARS, as shown in Figure 1. The time-dependence of TBARS production was examined (Figure 2) in the absence (line b) as well as in the presence of 0.5 mM H_2O_2 (line a). H_2O_2 in the absence of $V_{(IV)}$ was without effect. Exogenous H_2O_2 in the presence of $V_{(IV)}$ markedly increased the rate of formation of TBARS from DR. This is consistent with the rapid oxidation of $V_{(IV)}$ by H_2O_2 and with the rapid oxidation of NADH by $V_{(IV)}$ + H_2O_2.[6] $V_{(IV)}$ thus acts much as did Fe(II).[17] The ability of $V_{(IV)}$ to cause the degradation of DR was apparently dependent upon H_2O_2 generated during the autoxidation of $V_{(IV)}$. Thus, as shown in Figure 3, catalase inhibited. Moreover, scavengers of HO·, such as ethanol or HEPES,[18-19] also inhibited; indicating that HO·, produced from the reduction of H_2O_2 by $V_{(IV)}$, was an essential intermediate. $V_{(V)}$, which was itself inactive, did not gain activity in the presence of exogenous H_2O_2 and did not interfere with the activity of $V_{(IV)}$.

The optical spectrum of the TBARS generated during the degradation of deoxyribose by $V_{(IV)}$ is shown in Figure 4A. It is clearly similar to the product of the attack by the xanthine oxidase + xanthine + Fe(III) reaction mixture, which is known to generate HO·[14,17] on DR (Figure 4B). The TBARS formed from the attack of $V_{(IV)}$ on DR, in the presence of exogenous H_2O_2, gave the same spectrum as that seen in the

TABLE I
Vanadate-catalyzed Destruction of Deoxyribose in the Presence of Reducing Agents

Additions	A_{532}
$V_{(V)}$	0.072
NADH	0.060
$V_{(V)}$ + NADH	0.258
DTT	0.038
$V_{(V)}$ + DTT	0.147
cysteine	0.038
$V_{(V)}$ + cysteine	0.130

Reaction mixtures contained 3.5 mM DR in 25 mM sodium phosphate buffer, pH 7.4. Additions: 0.2 mM $V_{(V)}$; 1 mM NADH; 0.2 mM dithiothreitol (DTT); 0.2 mM cysteine.

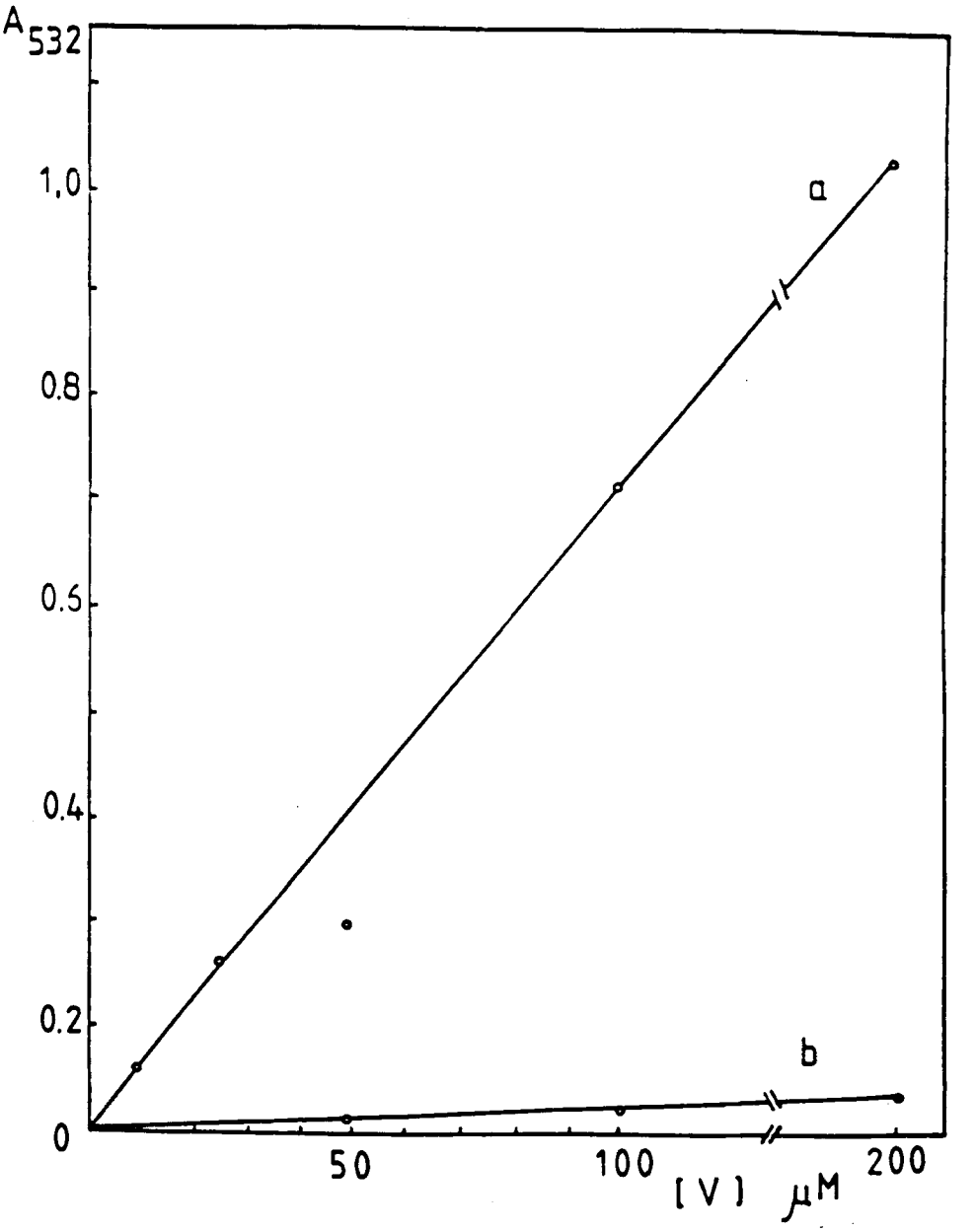

FIGURE 1 Dose effect of vanadium ions on the degradation of DR. 3.5 mM DR was incubated with vanadyl (a) or vanadate (b) in 25 mM phosphate buffer, pH 7.4 at 37°C for 30 min.

absence of exogenous H_2O_2. Fe(III), *per se*, did not cause detectable degradation of DR. Incubation of $V_{(V)}$ with DR led to the formation of a yellow color after development with thiobarbituric acid. This substance exhibited a peak absorbance at 450 nm, as shown in Figure 4A (line 2). The same yellow color and 450 nm absorption

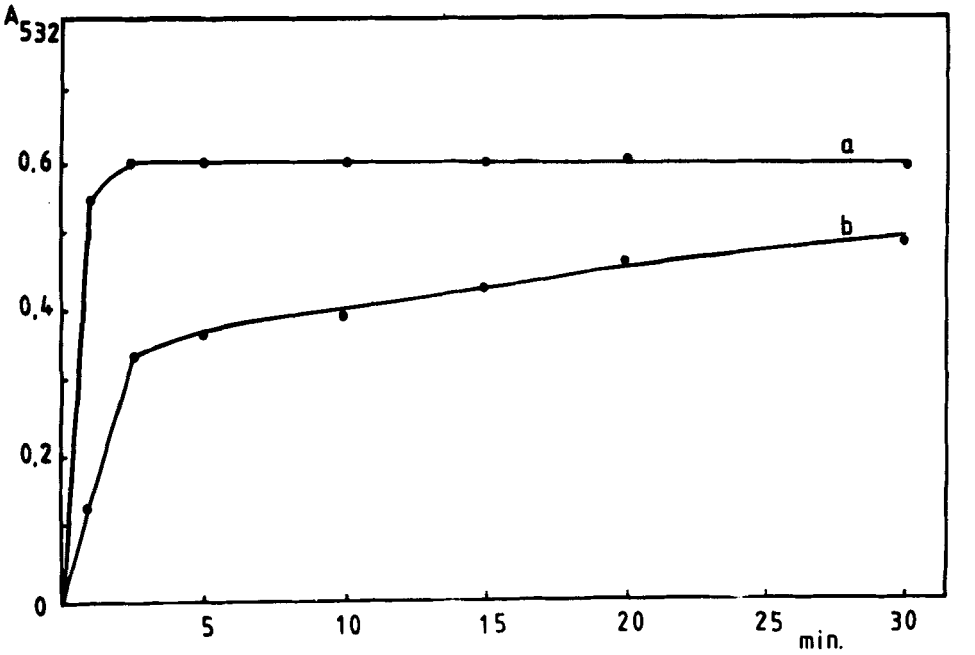

FIGURE 2 Time-dependence of HO· production by $V_{(IV)}$ in the presence (a) and in the absence (b) of H_2O_2. 3.5 mM DR was incubated with 0.1 mM $V_{(IV)}$ in 25 mM phosphate buffer, pH 7.4. H_2O_2 was present at 0.5 mM.

band were seen when $V_{(V)}$ and DR were added to the TBA reagent without prior incubation.

Reducing agents empowered $V_{(V)}$ to cause the degradation of DR, presumably by converting the $V_{(V)}$ to $V_{(IV)}$. The data in Table I demonstrate that NADH, dithiothreitol and cysteine were all effective, with NADH being the most active.

As expected,[14,17] addition of Fe(III) to the xanthine + xanthine oxidase reaction increased its ability to attack DR. The activity of the xanthine oxidase system in the

TABLE II
Effects of Vanadate on the Vanadyl- and Xanthine/Xanthine Oxidase-catalyzed Destruction of Deoxyribose

Additions	A_{532}
XO	0.189
0.2 mM $V_{(V)}$	0.067
XO + 0.2 mM $V_{(V)}$	0.092
XO + 0.1 mM $V_{(V)}$	0.120
XO + Fe^{3+}	0.459
XO + Fe^{3+} + 0.2 mM $V_{(V)}$	0.168
$V_{(IV)}$	0.303
$V_{(IV)}$ + 0.2 mM $V_{(V)}$	0.209

Reaction mixtures contained 0.3 mM xanthine and 3.5 mM DR in 25 mM sodium phosphate buffer, pH 7.4. Additions: 50 μg/ml xanthine oxidase (XO); 0.1 mM $V_{(IV)}$; 10 μM Fe^{3+}; 0.1 or 0.2 mM $V_{(V)}$.

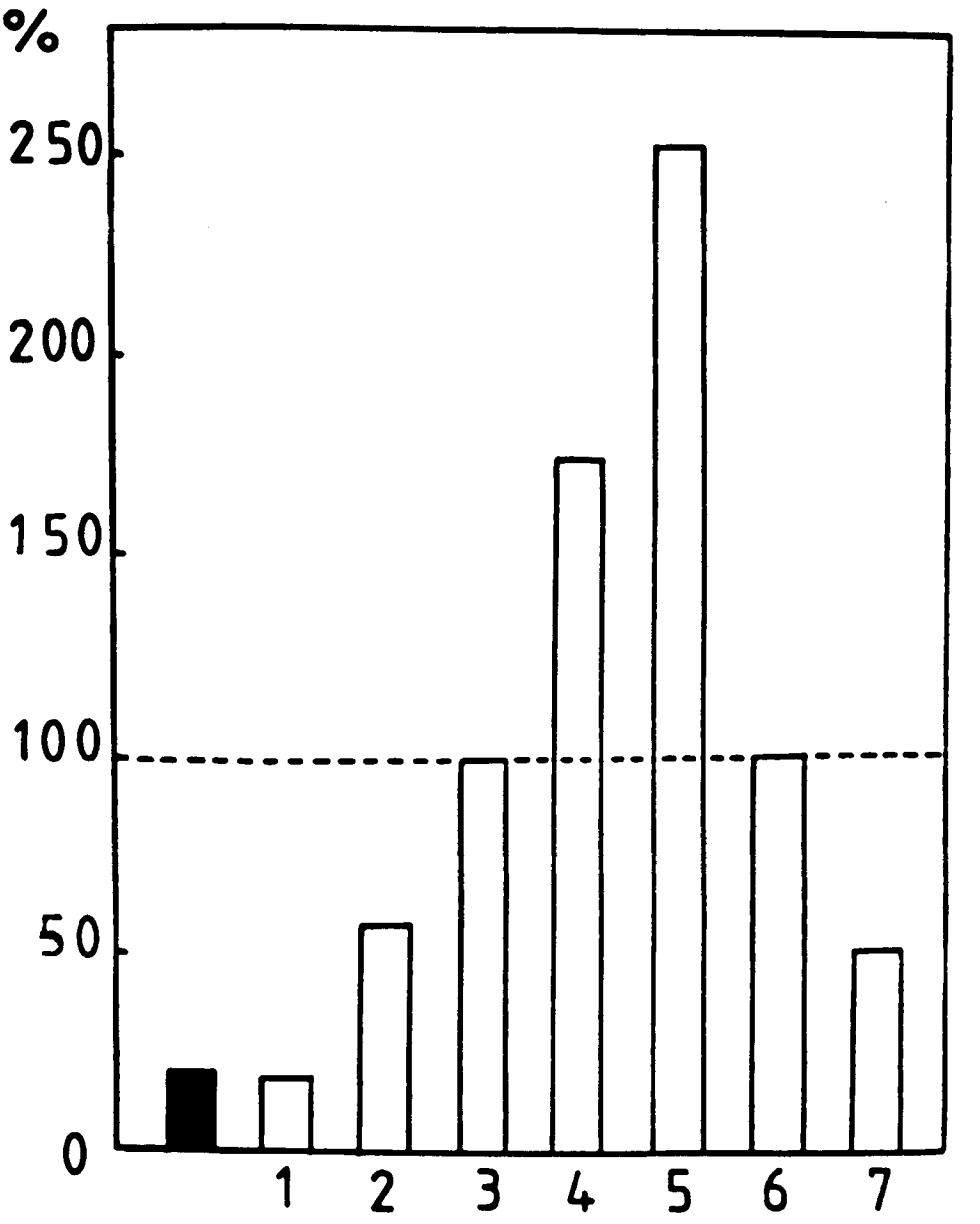

FIGURE 3 Effect of active oxygen species scavengers and other agents on $V_{(IV)}$-dependent production of HO·. 3.5 mM DR was incubated with 0.1 mM $V_{(IV)}$ in 25 mM phosphate buffer (open bars) or in 25 mM HEPES buffer (dark bar), pH 7.4. Additions: 1 – catalase (20 μg/ml); 2 – 1 % ethanol; 3 – SOD (10 μg/ml); 4 – heat-denatured catalase (20 μg/ml); 5 – Fe^{3+} (10 μM); 6 – urate (200 μM); 7 – 0.3 mM xanthine. The dashed line denotes control (A_{532} = 0.622).

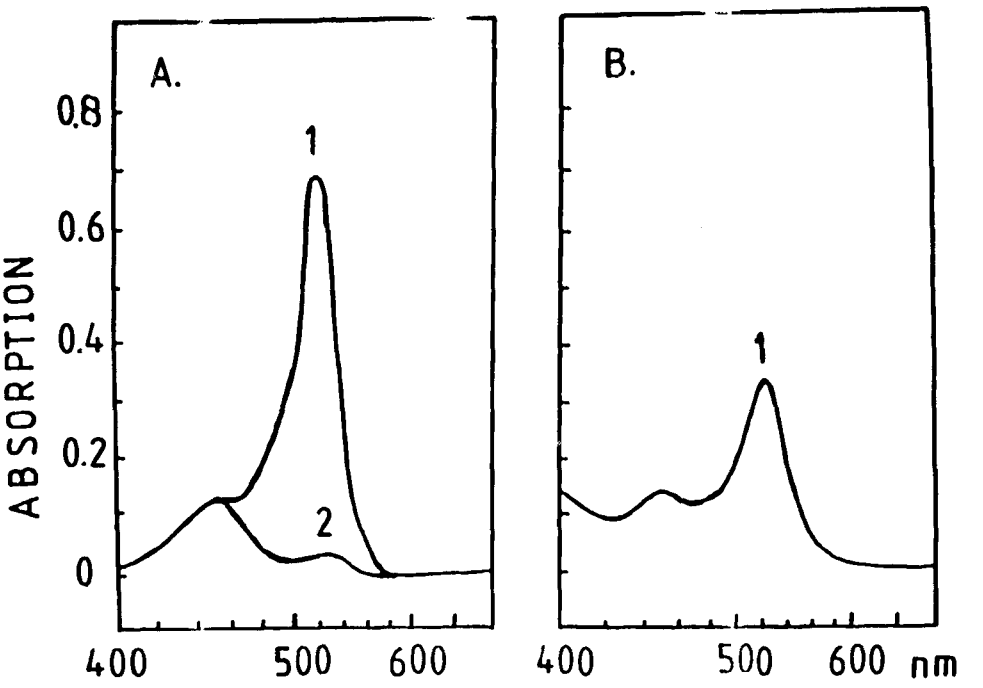

FIGURE 4 Spectra of TBARS formed upon degradation of DR by vanadium ions (A) and by xanthine/xanthine oxidase/Fe^{3+} system (B). 3.5 mM DR was incubated in 25 mM phosphate buffer, pH 7.4. Additions: *A* – 0.1 mM $V_{(IV)}$ (1); 0.1 mM $V_{(V)}$ (2); *B* – 0.3 mM xanthine; 10 μM Fe^{3+}; 50 μg/ml xanthine oxidase.

absence of added Fe(III) was probably due to endogenous trace metals. $V_{(V)}$, in contrast, did not exert this effect, as shown by the data in Table II. Indeed $V_{(V)}$ decreased the attack by the xanthine + xanthine oxidase reaction on DR. This was not simply due to inhibition of the xanthine oxidase reaction since these levels of $V_{(V)}$ diminished the rates of urate production, or of cytochrome *c* reduction, by less than 10 %.

DISCUSSION

The results presented above can be accommodated by a reaction scheme in whic the gradual autoxidation of $V^{2.6}_{(V)}$ yields O_2^-, which dismutes to H_2O_2. $V_{(IV)}$ then reduces this endogenous H_2O_2 to yield HO·, which attacks DR leading to carbonyl products reactive with TBA. Thus:

$$\text{(a) } V_{(IV)} + O_2 \rightharpoonup V_{(V)} + O_2^-$$

$$\text{(b) } O_2^- + O_2^- + 2H^+ \rightharpoonup H_2O_2 + O_2$$

$$\text{(c) } H_2O_2 + V_{(IV)} \rightleftharpoons HO^- + HO\cdot + V_{(V)}$$

$$\text{(d) } HO\cdot + DR \rightarrow\rightarrow \text{TBARS}$$

Compounds such as ethanol or HEPES compete with DR for the HO· and thus block the production of TBARS. Catalase inhibited by removal of H_2O_2: whereas SOD was without effect, because reaction (b) was not rate-limiting and the only role of O_2^- was as a precursor of H^2O_2 which would be produced by either the spontaneous, or the SOD-catalyzed dismutation reaction. Halliwell *et al.*[17] have proposed a similar mechanism for the Fe(II)-catalyzed oxidation of *DR*.

$V_{(V)}$ was ineffective, unless reductants were present. NADH or thiols activated the $V_{(V)}$ by reducing it to $V_{(IV)}$. The formation of HO· in a $V_{(V)}$ plus thiol reaction mixture has been demonstrated by spin-trapping.[20] The inability of $V_{(V)}$ to augment the co-oxidation of DR by the xanthine oxidase reaction is at odds with results reported by Keller *et al.*[4] Using spin-trapping these authors described a $V_{(V)}$-dependent productin of HO· by the xanthine oxidase reactin and proposed that O_2^-, generated by this enzymatic reaction, was able to reduce $V_{(V)}$ to $V_{(IV)}$. Were that the case we should have seen $V_{(V)}$-dependent increase in the conversion of DR to TBARS. To the contrary, we noted that $V_{(V)}$ diminished the co-oxidation of DR by the xanthine oxidase reaction. The reason for this effect of $V_{(V)}$ remains to be explored.

Since cells contain reductants, such as thiols and NAD(P)H, $V_{(V)}$ will readily be converted to $V_{(IV)}$ upon entering cells.[1,3] Our results suggest that this $V_{(IV)}$ may then lead to the production of HO· which could contribute to the toxicity of vanadium salts.

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References

1. D.W. Boyd and K. Kustin, (1985) Vanadium: a versatile biochemical effector with an elusive biological function. *Advances in Inorganic Biochemistry*, **6**, 311–365.
2. S. Liochev and I. Fridovich (1990) Vanadate-stimulated oxidation of NAD(P)H in the presence of biological membranes and other sources of O_2^-. *Archives in Biochemistry and Biophysics*, **279**, 1–7.
3. B.R. Nechai (1984) Mechanisms of action of vanadium. *Annual Reviews in Pharmacological Toxicology*, **24**, 501–524.
4. R.J. Keller, R.A. Coulombs, R.F. Sharma, T.A. Grover and L.H. Piette (1989) Importance of radical in the vanadium stimulated oxidation of NADH. *Free Radicals in Biology Medicine*, **6**, 15–22.
5. R.J. Keller, R.P. Sharma, T.A. Grover and L.H. Piette (1988) Vanadium and lipid preoxidation. Evidence for involvement of vanadyl and hydroxyl radical. *Archives in Biochemistry and Biophysics*, **265**, 524–532.
6. S. Liochev and I. Fridovich (1987) The oxidation of NADH by tetravalent vanadium. *Archives in Biochemistry and Biophysics*, **255**, 274–278.
7. J. Kuwahara, T. Suzuki and Y. Sugiura. (1985) Effective DNA cleavage by bleomycin-vanadium (IV) complex plus hydrogen peroxide. *Biochemistry and Biophysics Research Communications*, **129**, 368–374.
8. G.M. Woltermann, R.H. Scott and G.P. Haig (1974) Coupling of adenosine triphosphate hydrolysis to a simple inorganic redox system vanadyl ion plus hydrogen peroxide. *Journal of the American Chemistry Society*, **95**, 5569–5570.
9. T. Ozawa and A. Hanaki (1989) ESR evidence for the formation of hydroxyl radicals during the reaction of vanadyl ions with hydrogen peroxide. *Chemistry and Pharmaceutical Bulletin*, **37**, 1407–1409.
10. S. Liochev and E. Ivancheva (1988) A study on the mechanism of the vanadate-dependent NADH oxidation. *Free Radicals in Biology Medicine*, **5**, 349–354.
11. A.J. Carmichael (1990) Vanadyl-induced Fenton-like reaction in RNA. An ESR and spin trapping study. *FEBS Letters*, **261**, 165–170.
12. S. Liochev and I. Fridovich (1989) Hydroxyl radical is not a significant intermediate in the vanadate-simulated oxidation of NAD(P)H by O_2^-. *Archives Biochemistry and Biophysics*, **275** 40–43.

13. S. Liochev, E. Ivancheva, and E. Russanov (1988) Vanadyl- and vanadate-induced lipid peroxidation in mitochondria and in phosphatidylcholine suspension. *Free Radical Research Communications*, **4**, 317–323.
14. K. Brawn and I. Fridovich (1981) DNA strand scission by enzymically generated oxygen radicals. *Archives Biochemistry and Biophysics*, **206**, 414–419.
15. S. Vijaya, F.L. Crane and Ramasarma (1984) A vanadate-stimulated NADH oxidase in erythrocyte membranes generates hydrogen peroxide. *Molecular Cellular Biochemistry*, **62**, 175–185.
16. T.A. Grover and L.H. Piette (1981) Influence of flavin addition and removal in the formation of superoxide by NADPH-cytochrome P-450 reductase: a spin-trap study. *Archives in Biochemistry and Biophysics*, **212**, 105–114.
17. B. Halliwell, M. Grootveld and J.M.C. Gutteridge (1988) Methods for the measurement of hydroxyl radicals in biochemical systems: deoxyribose degradation and aromatic hydroxylation. *Methods in Biochemical Analysis*, **33**, 59–90.
18. W. Pashen and U. Weser (1975) Problems concerning the biochemical action of superoxide dismutase (erythrocuprein). *Hoppe-Seyler's z. Physiol. Chem.*, **356**, 727–737.
19. B. Halliwell and S. Ahluwala (1976) Hydroxylation of p-coumaric acid by horse-radish peroxidase. *Biochemical Journal*, **153**, 513–518.
20. R.J. Keller, R.A. Coulombe, R.P. Sharma, T.A. Grover, and L.H. Piette (1989) Oxidation of NADH by vanadium compounds in the presence of thiols. *Archives in Biochemistry and Biophysics*, **271**, 40–48.

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