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Interference by metavanadate in the assay for formaldehyde and 4-aminophenol

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Vanadium compounds have been used for a number of years to test their effect on biological systems as they interfere with the use of phosphate (because of the similarity of the vanadate and phosphate ions) [1, 2] and with redox reactions within cells (due to the ability of vanadium to readily change its valence state) [3, 4]. The latter effect of vanadium compounds has led to studies of their effects on hepatic drug metabolism. For example, *in vivo* treatment with vanadium (III) chloride has been shown to inhibit hydroxylation of benz(*a*)pyrene [5] whereas metavanadate has no effect on aminopyrine metabolism *in vitro* [6]. Beyhl [7] has shown that ammonium metavanadate (NH_4VO_3) inhibits the metabolism of many drug substrates when added directly to preparations of hepatic microsomes. Donahue and Morgan [8] have shown that *in vivo* treatment of diabetic male rats with sodium metavanadate can reverse some of the effects of the diabetes on hepatic levels of cytochrome P-450 with a marked decrease in the level of cytochrome P-450j by metavanadate (reversing an eightfold rise induced by diabetes) but with no change in cytochrome P-450h (which showed a marked fall in the diabetic state). 4-Nitrophenol hydroxylase activity followed the levels of cytochrome P-450j in these studies.

In our own studies on the interaction of sodium metavanadate with hepatic drug metabolism, we noticed some disrepancies in the effects noted *in vivo* and those seen with the *in vitro* addition of the vanadium compound [9]. In order to clear up these anomalies, we decided to investigate the effect of metavanadate on the assays for the metabolic products being used in our studies.

Materials and methods

Chemicals. Sodium metavanadate (90%) was puchased from the Aldrich Chemical Co. (Gillingham, U.K.) and all other chemicals were of the highest purity available commercially. Aniline was repurified before use and stored in opaque containers to avoid photo-oxidation.

Assays. Two assays were investigated in this study; the measurement of 4-aminophenol (a major metabolite of aniline produced by hepatic microsomes) and the measurement of formaldehyde (a very common assay used to determine the rate of demethylation of many drug substrates). 4-Aminophenol was assayed by a modification of the method of Schenkman *et al.* [10] and involved mixing a solution containing the 4-aminophenol with an alkaline phenol solution which produces a coloured phenol-indophenol complex (λ_{max} at 630 nm). Formaldehyde was assayed by the method of Nash [11] using the Hantzsch reaction (formaldehyde, acetylacetone and ammonia condense to form 3,5-diacetyl-1,4-dihydrolutidine). The condensation product has an λ_{max} at 415 nm. Spectrophotometric measurements were made using a Shimadzu UV-240 dual beam spectrophotometer with the relevant blank solution in the reference cuvette. Sodium metavanadate dissolved in distilled water was added at the appropriate concentration where indicated. Distilled water in a similar amount was added to the control assays.

Analysis of results was performed using the paired *t*-test employing a custom-made computer program. The level of significance was set at $P < 0.05$.

Results and discussion

Figure 1 shows the results for the assay of 4-aminophenol in the presence and absence of 2–10 mM sodium metavanadate. It is seen that in the absence of metavanadate the response of the assay to 4-aminophenol was linear over the entire range of concentrations (0–50 nmol/assay) used. In the presence of sodium metavanadate, however, the response to 4-aminophenol was markedly diminished and was no longer linear. Indeed, at the higher concentrations of metavanadate (5 and 10 mM), there was virtually no response in the assay to 4-aminophenol even at the highest concentration (50 nmol/assay). At 50 nmoles of 4-aminophenol/assay there was a 74% inhibition of the response at 2 mM metavanadate and a 95% inhibition at 5 mM metavanadate.

A similar inhibition was seen for the assay of formaldehyde (Fig. 2). In the absence of metavanadate, a linear response of formaldehyde was seen (up to 100 nmol fomaldehyde/assay). In the presence of metavanadate, however, an inhibited and non-linear response was seen. The effect of metavanadate, as with the assay for 4-aminophenol, appeared to be dose-dependent with a more marked inhibition of the reaction at 10 mM metavanadate than that seen with the lower concentrations. There appeared to be less inhibition of the reaction at higher concentrations of formaldehyde (e.g. 89% inhibition at 60 nmol formaldehyde/assay with 10 mM metavanadate but only 41% inhibition at 100 nmol formaldehyde/assay with the same concentration of metavanadate).

The presence of metavanadate (as the sodium salt) in the assay for 4-aminophenol and formaldehyde by the colorimetric procedures described above, therefore, has a marked effect on the linearity and sensitivity of the assay and it is doubtful whether the assays are valid in the presence of the concentrations of metavanadate used in this study.

This study casts some doubt on the results obtained by other workers investigating the effects of vanadium compounds *in vitro* such as that of Beyhl [6, 7] where up to 1 mM metavanadate was used. The study of Beyhl [7]

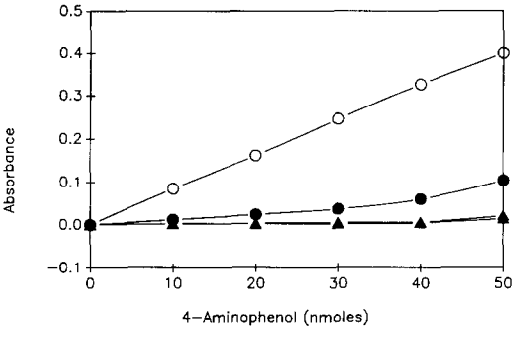

Fig. 1. Assay of 4-aminophenol in the absence (—○—) or presence of 2 mM (—●—), 5 mM (—△—) or 10 mM (—▲—) sodium metavanadate. All curves in the presence of metavanadate were significantly different ($P < 0.05$) from the control curve. Each point represents four samples and the SD was less than the size of the symbols.

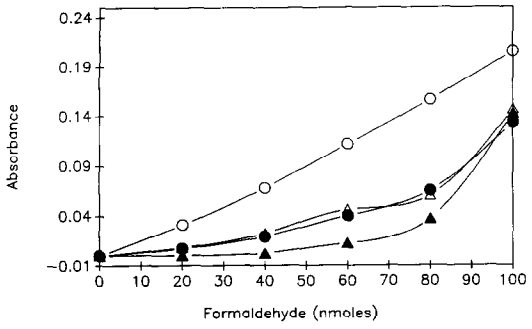

Fig. 2. Assay of formaldehyde in the absence (—○—) or presence of 2 mM (—●—), 5 mM (—△—) or 10 mM (—▲—) sodium metavanadate. All curves in the presence of metavanadate were significantly different from the control curve ($P < 0.05$). Each point is an average of four samples and the SD was less than the symbol size in all cases.

is particularly interesting in that both colorimetric and fluorimetric assays were used and both types of assay indicated inhibition of enzyme activity. This may indicate that fluorimetric, as well as colorimetric, assays may be affected by metavanadate. It is also interesting to note that aminopyrine N-demethylation, assayed by formaldehyde measurement, appeared to be unaffected whereas anisic ester and papaverine O-demethylation, measured by the same method, were reported to be inhibited. This could indicate that metavanadate is having no effect in this study on the measurement of formaldehyde or that metavanadate does indeed inhibit the assay of the metabolites but the effect on some substrates is masked by a concomitant increase in activity of the specific enzyme by metavanadate, an effect noted by ourselves [9] using *in vivo* treatment with metavanadate. None of the papers indicate that this potential problem has been noticed and, indeed, it would simply appear as an inhibition of the metabolism of aniline or drug substrates that could be demethylated. A similar problem could exist if vanadium compounds administered *in vivo* were carried through to the preparation used in determining hepatic drug metabolism. Work is in progress to determine the amount of metavanadate found in hepatic microsomes following *in vivo* treatment with the compound over an extended period of time.

It is clear that metavanadate can have other effects on drug metabolism apart from inhibition of assay of metabolites as shown by Donahue and Morgan [8] and Halliday and Skett [9] using *in vivo* vanadate treatment.

In summary, extreme care should be used when attempting to interpret data obtained from experiments designed to investigate the effects of vanadium compounds on hepatic drug metabolism where colorimetric assays are used as a measure of drug-metabolising capacity, particularly when the compounds are added *in vitro*.

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REFERENCES

1. Cantley LA, Josephson L, Warner R, Yanagisawa M, Lechene C and Guidotti G, Vanadate is a potent (Na,K)-ATPase inhibitor found in ATP derived from muscle. *J Biol Chem* **252**: 7421–7423, 1977.
2. Schwabe U, Puchstein C, Hannemann H and Söchtig E, Activation of adenylate cyclase by vanadate. *Nature* **277**: 143–145, 1979.
3. DeMaster EG and Mitchell RA, A comparison of arsenate and vanadate as inhibitors or uncouplers of mitochondrial and glycolytic metabolism. *Biochemistry* **12**: 3616–3621, 1973.
4. Byczkowski JZ, Zychlinksi L and Tluczkiewicz J, Interaction of vanadate with respiratory chain of rat liver and wheat seedling mitochondria. *Int J Biochem* **10**: 1007–1011, 1979.
5. Calop J, Burckhart M-F and Fontanges F, Etude de l'influence des éléments traces sur l'hydroxylation du benzo(*a*)pyrene. *Eur J Toxic Environ Hyg* **9**: 271–286, 1976.
6. Beyhl FE, Ammonium metavanadate—an inhibitor of hepatic microsomal monooxygenases. *IRCS Med Sci* **9**: 545, 1981.
7. Beyhl FE, Action of ammonium metavanadate on the activities of hepatic drug-metabolizing enzymes *in vitro*. *Biochem Pharmacol* **31**: 1458–1461, 1982.
8. Donahue BS and Morgan ET, Effects of vanadate on expression of hepatic cytochromes P-450j and P-450h in streptozotocin-diabetic rats. *Biochem Biophys Res Commun*, in press.
9. Halliday SE and Skett P, The effects of vanadate on hepatic drug metabolism in normal and streptozotocin-diabetic rats. *Biochem Pharmacol*, in press.
10. Schenkman JB, Remmer H and Estabrook RW, Spectral studies of drug interaction with hepatic microsomal cytochrome P-450. *Mol Pharmacol* **3**: 113–123, 1967.
11. Nash T, The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* **55**: 416–421, 1953.