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# Proliferative and morphological changes induced by vanadium compounds on Swiss 3T3 fibroblasts

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**Vanadium compounds are shown to have a mitogenic effect on fibroblast cells. The effects of vanadate, vanadyl and pervanadate on the proliferation and morphological changes of Swiss 3T3 cells in culture are compared. Vanadium derivatives induced cell proliferation in a biphasic manner, with a toxic-like effect at doses over 50 μM, after 24 h of incubation. Vanadyl and vanadate were equally potent at 2.5–10 μM. At 50 μM vanadate inhibited cell proliferation, whereas slight inhibition was observed at 100 μM of vanadyl. At 10 μM pervanadate was as potent as vanadate and vanadyl in stimulating fibroblast proliferation, but no effect was observed at lower concentrations. A pronounced cytotoxic-like effect was induced by pervanadate at 50 μM. All of these effects were accompanied by morphological changes: transformation of fibroblast shape from polygonal to fusiform; retraction with cytoplasm condensation; and loss of lamellar processes. The magnitude of these transformations correlates with the potency of vanadium derivatives to induce a cytotoxic-like effect: pervanadate > vanadate > vanadyl. These data suggest that the oxidation state and coordination geometry of vanadium determine the degree of the cytotoxicity.**

**Keywords:** cytotoxicity, fibroblasts, growth factors, proliferation, vanadium

## Introduction

Vanadium is a trace transition metal which has been shown to have insulin-mimetic actions on mammalian cells in culture (Smith 1983, Lau *et al.* 1988, Mountjoy & Flier 1990, Wang & Scott 1992, 1994, Chen & Chan 1993, Cortizo & Etcheverry 1995). Two kinds of vanadium effects have been described in cultured cells: metabolic or short time actions; and mitogenic or chronic effects (Schechter 1990, Schechter & Shisheva 1993). Vanadate, one of the major chemical forms of vanadium in living systems, has been postulated to exert its actions via the inhibition of tyrosine phosphatase (PTPase) (Gresser *et al.* 1987, Gresser & Tracey 1990). By binding to their specific receptors, growth factors induce phosphorylation of these receptors and thereby promote cell proliferation (Lau *et al.* 1989).

The bioactivity of different vanadium compounds (V (V) and V (IV) derivatives) has been tested on several cell types. In BALB/3T3 cells it has been demonstrated that vanadate shows strong cytotoxicity and causes morphological transformation of the cells (Sabbioni *et al.* 1993). On the other hand, vanadium (IV) shows a less toxic effect in this cell line (Sabbioni *et al.* 1991). It has been shown by electron paramagnetic resonance (EPR) spectroscopy that vanadate reduces to vanadyl inside the cells, this being a protective mechanism to avoid cytotoxicity (Sabbioni *et al.* 1992). Pervanadates, a group of vanadium (V) derivatives, have shown stronger effects than vanadate in adipocytes (Fantus *et al.* 1989, Lönnroth *et al.* 1993, Shisheva & Shechter 1993), HL-60 cells (Bourgoin & Grinstein 1992), skeletal muscle (Leighton *et al.* 1991) and UMR106

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cells (Cortizo & Etcheverry 1995). Nevertheless, pervanadates may have toxic effects on certain cellular types.

The aim of this study was to compare the mitogenic effect and morphological changes induced on Swiss 3T3 fibroblasts by different vanadium compounds.

## Materials and methods

*Materials*

Vanadium (IV)-oxide sulfate (vanadyl sulfate) was obtained from Merck (Darmstadt, Germany), *o*-vanadate, catalase and *p*-nitrophenylphosphate (pNPP) were obtained from Sigma (St. Louis, MO, USA). Tissue culture material was provided by Corning (Princeton, NJ, USA). Dulbecco's modified Eagle's medium (DMEM) and trypsin were supplied by Gibco (Gaithersburg, MD, USA) and fetal bovine serum (FBS) by Gen (Buenos Aires, Argentina).

*Vanadium compounds and solution preparations*

Vanadate solution (100 mM) was prepared from sodium *o*-vanadate (Sigma). Vanadium (IV)-oxide sulfate (vanadyl sulfate) was obtained from Merck and dissolved in distilled water to obtain a 100 mM fresh solution. In the concentration range used (2.5–100 μM) the culture medium has enough buffer capacity to maintain a pH value of 7.4. Pervanadate was prepared according to Trudel *et al.* (1991). Briefly, equivalent amounts of 10 mM *o*-vanadate and 10 mM $H_2O_2$ were mixed at room temperature for 15 min. Then, 200 μg $ml^{-1}$ catalase were added to eliminate the excess $H_2O_2$. This procedure resulted in the generation of the peroxidized form of vanadate, which is stable for 2 h without further addition of $H_2O_2$ (Kadota *et al.* 1987). Controls for these experiments consisted of a similar preparation in the absence of vanadate. No statistical differences were found in comparison with the cells incubated in DMEM alone (basal conditions). All the solutions were added to the culture medium immediately after their preparation.

*Cell culture*

Swiss 3T3 cells were cultured in DMEM supplemented with 100 U $ml^{-1}$ penicillin, 100 μg $ml^{-1}$ streptomycin and 10% FBS in a humidified atmosphere of 95% air/5% $CO_2$. Cells were subcultured by trypsin treatment when they became 70% confluent. For experiment, about $2.5 \times 10^4$ cells per ml were plated into 24-well plates. After the cells had reached 70% confluence, the monolayer was washed with DMEM without serum and incubated in 0.5 ml DMEM plus various concentrations of vanadium compounds for 24 h.

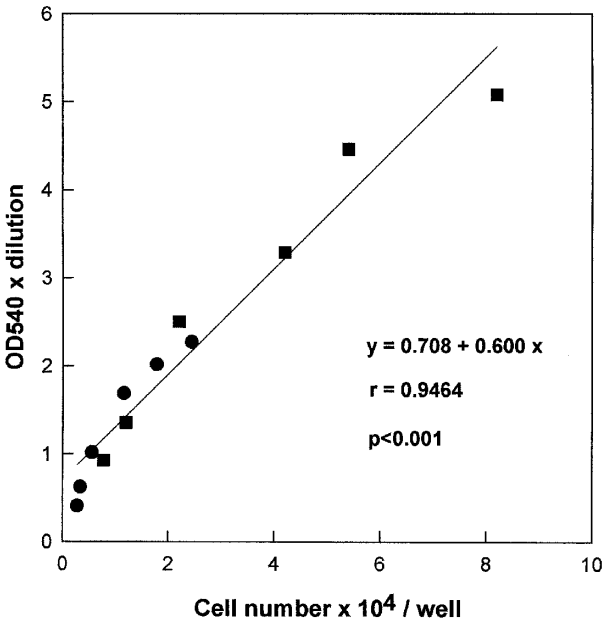


**Figure 1.** Correlation between the total cell number per well and the optical density at 540 nm corrected by dilution of cell extract (crystal violet assay). ■,● values represent two independent experients performed by triplicate.

*Cell proliferation assay*

A mitogenic bioassay was carried out as described previously (Cortizo & Etcheverry 1995). The crystal violet fixed by cells was quantified at 540 nm after an extraction procedure. In order to confirm that the colorimetric bioassay correlated with cell proliferation, the relationship between cell number per well and the absorbance at 540 nm was examined. Cell number was determined by trypsinization of wells and counting the cells with a Neubauer chamber. Figure 1 demonstrates a strong linear correlation between the total cell number per well and the absorbance at 540 nm corrected by sample dilution over the range of cell numbers obtained by stimulation with different agents. In the experiments, cells were incubated with different vanadium compounds at the doses and periods indicated in the figure legends.

*Morphology*

Cells were plated into a 35 mm dish at a density of $2 \times 10^4$ cells per dish. After 24 h, cells were washed with DMEM and incubated in fresh DMEM plus different vanadium compounds (50 μM) for an additional 24 h. Cells were then washed and stained with crystal violet as before and the morphological changes were examined.

*Statistical methods*

Data are expressed as the mean ± SEM. Statistical differences were analysed using Student's t-test or analysis of

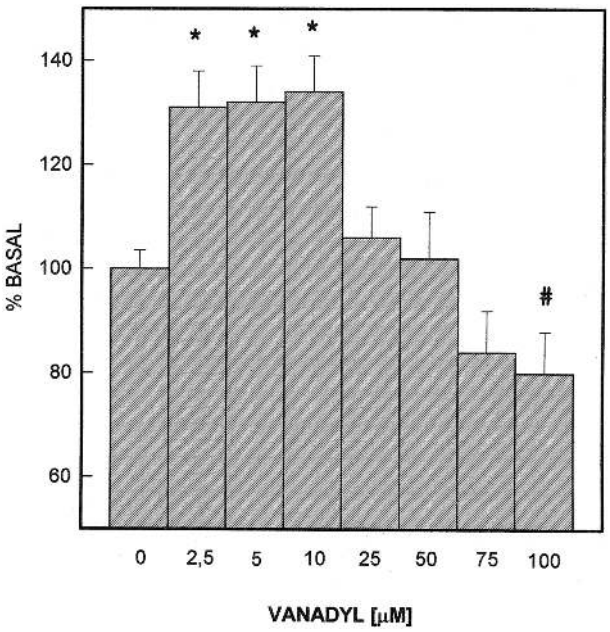


**Figure 2.** Effect of vanadyl cation on cell proliferation. Data are expressed as % over basal and indicate the means ± SEM ($n$ = 12). $P$ values versus basal are: # $P < 0.05$, *$P < 0.001$.

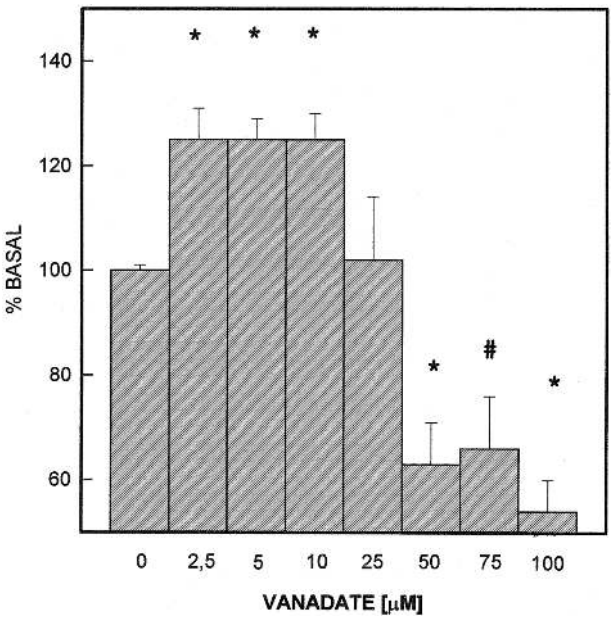


**Figure 3.** Effect of vanadate on cell proliferation. Data are expressed as % over basal and indicate the means ± SEM ($n$ = 12). $P$ values versus basal are: # $P < 0.01$, *$P < 0.001$.

variance when suitable. Linear regression analysis was performed by Pearson's correlation coefficient.

## Results

### *Effect of vanadium compounds on cell proliferation*

To assess the effect of vanadium derivatives on fibroblast proliferation, the crystal violet bioassay was performed. All vanadium compounds stimulated cell progression in a narrow range of concentration after 24 h of culture.

Vanadyl increased Swiss 3T3 fibroblast proliferation in a biphasic manner (Figure 2). Low doses (2.5–10 μM) significantly increased cell growth in the range 131–134% of basal ($P < 0.001$). No effect was observed at higher concentrations (up to 100 μM). At the 100 μM dose, a slight but significant inhibition was observed (80% of basal, $P < 0.05$).

The incubation of cells with vanadate induced cell proliferation in a biphasic manner, like vanadyl, with a maximum between 2.5 and 10 μM (125% of basal, $P < 0.001$) (Figure 3). However, doses between 50 and 100 μM strongly inhibited cell proliferation (63–54% of basal, $P < 0.01$–$0.001$).

Pervanadate caused stimulation of cell growth at 10 μM (118% of basal, $P < 0.01$) (Figure 4). Its main effect was a dose-response inhibition on mitogenesis between 50 and 100 μM (33–9% of basal, $P < 0.001$).

The effectiveness of vanadium compounds in stimulating cell proliferation was analysed. At low doses (2.5 and 5 μM) vanadate and vanadyl, but not pervanadate, were similarly potent mitogens. However, the 10 μM concentration of pervanadate induced a stimulation statistically comparable with the other two derivatives. On the other hand, comparison of the toxic-like effect, as assessed by cell growth inhibition, suggests that pervanadate was the most toxic agent. At 50 μM it inhibited 70% of the basal proliferation, whereas the effect of vanadate was only 37% ($P < 0.01$, pervanadate versus vanadate). Under similar conditions no effect was observed with the vanadyl cation.

### *Morphological changes induced by vanadium compounds*

In the Swiss 3T3 cells exposed to 50 μM of vanadium compounds, the extent of the morphological changes in cells was evident after 24 h.

Control cultures showed polyhedrical or stellate fibroblasts with slender lamellar expansions (Figure 5A). These expansions appeared to be joined to each other among neighbour cells. The nuclei showed

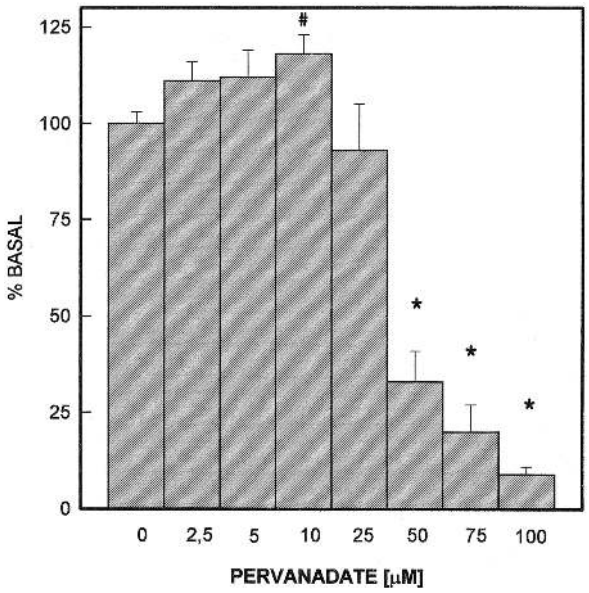


**Figure 4.** Effect of pervanadate on cell proliferation. Data are expressed as % over basal and indicate the means ± SEM ($n = 9$). $P$ values versus basal are: # $P < 0.01$, *$P < 0.001$.

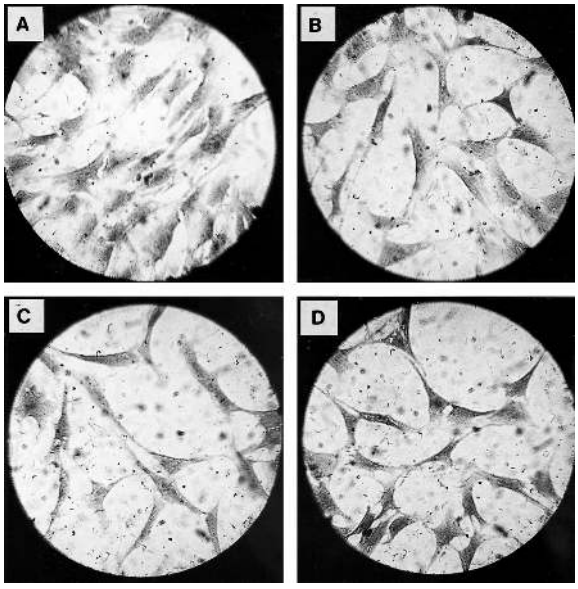


**Figure 5.** Effect of vanadium compounds on Swiss 3T3 fibroblast morphology. Cells were incubated in DMEM (control, A), or μM of vanadyl (B), vanadate (C) or pervanadate (D) for 24 h. After this period, cells were stained with crystal violet. Objective × 40.

moderately thick chromatin granules. The perinuclear cytoplasm had numerous organelles and vacuoles, while the exoplasm was diffuse and homogeneous.

The fibroblasts from vanadyl-treated cultures displayed slight changes with respect to those from control cultures (Figure 5B). In general, cells were less polyhedrical, more fusiform, showing denser and less extended cytoplasmic processes. The nucleus contained less defined chromatin granules.

Cells cultured with vanadate showed clear morphological changes (Figure 5C). Fibroblasts were fusiform in shape, had a more condensed cytoplasm and less processes, with clearly defined borders. They seemed to be smaller than the fibroblasts from control cultures. A few stellate-shaped cells with lamellar expansions were also found in these cultures.

Cultures treated with pervanadate showed the most pronounced morphological changes (Figure 5D). Cells were long, fusiform, very dense, and presented well-defined borders. These fibroblasts displayed few and fine processes and showed a very dense cytoplasm.

These results seem to indicate that vanadium (V) derivatives are stronger morphological transformers for Swiss 3T3 fibroblasts than are vanadium (IV) compounds.

In addition, a morphological study was carried out at 10 μM concentration of the three vanadium derivatives. This dose was chosen because it induced the maximal stimulation of cellular proliferation. The results showed that cells incubated with vanadyl and vanadate were not different from the control cultures (results not shown). Cultures treated with 10 μM pervanadate showed a very few cells with more condensed cytoplasm, but with a general morphology similar to the control.

In order to get a deeper insight into the cytotoxicity of vanadium compounds, a time course of vanadate (50 μM) was carried out, studying in parallel the morphological and proliferative modifications. As early as 6 h of culture, a few fusiform cells could be seen among normal polygonal cells (results not shown). The monolayer incubated for 12 h with vanadate showed an increase in the proportion of fusiform cells without processes, and this tendency was more pronounced after 24 and 36 h. These experiments suggest that the morphological changes with 50 μM vanadate evolved as a function of the incubation time. On the other hand, the proliferative inhibition was only observed after 24 h of incubation (73% of basal, $P < 0.05$). No differences were found after 6 h (98% of basal) or 12 h (84% of basal) incubation with vanadate. This study suggests that the inhibition of cell growth is delayed in comparison with the morphological alterations.

## Discussion

It has been shown previously that vanadate stimulates DNA synthesis in several cell lines (Smith 1983, Mountjoy & Flier 1990, Davidai *et al*. 1992, Cortizo & Etcheverry 1995). This effect is supposed to be mediated by the inhibition of PTPases, which in turn regulate the levels of phosphoproteins. Thus, vanadate mimics the effects of insulin and insulin-like grown factors (Lau *et al*. 1989). The present study provides new experimental evidence for the proliferation and morphological transformation induced by vanadium compounds in fibroblasts in culture. The oxidation state and coordination geometry of vanadium in the different derivatives are also considered in relationship to their bioactivity.

The three vanadium derivatives tested showed a biphasic effect on Swiss 3T3 fibroblast growth. At low concentrations, the maximum stimulatory effect was obtained with 10 μM of the three vanadium compounds. On the other hand, vanadyl, vanadate and pervanadate behaved differently as inhibitors of cellular proliferation. At 50 μM concentration, vanadyl did not show cytotoxic-like effects, whereas vanadate and pervanadate strongly inhibited cell development. Furthermore, vanadate showed a weaker cytotoxic effect than pervanadate.

The speciation of the peroxo compounds formed under our experimental conditions were not determined. According to other authors, the species and structures in solution have not been characterized (Stankiewicz & Tracey 1995). Nevertheless, according to this reference, we think that the two predominant species in solution are mono- and diperoxovanadates. Diperoxovanadates interact with different ligands forming strong monodentate complexes. On the other hand, mono-peroxovanadates tend to generate multidentate products. However, the way in which this behaviour is reflected in the toxicity and other effects in the cell cultures is still unknown.

The observed effects of vanadium compounds on cellular proliferation were clearly associated with the morphological transformation of fibroblasts. We selected a concentration of 50 μM for these studies since at this dose, clear differences in cell proliferation were found among the three vanadium derivatives. In order to facilitate the observation of the morphological changes, cells were also plated on a 35 mm dish at a lower cell density than in the proliferation studies. Vanadium compounds induced the cells to display an unusual fusiform aspect, with few processes and condensed cytoplasm. These changes were more important in the case of pervanadate and vanadate; vanadyl showing the least effect. Other authors have previously demonstrated that vanadate induced phenotypic transformation in different cell lines (Klarlund 1985, Mountjoy & Flier 1990, Sabbioni *et al*. 1993); our studies confirm their results and demonstrate for the first time that pervanadate influences cellular morphology. Moreover, no direct evidence of a relationship between morphological and biochemical changes has previously been given. Our results suggest a direct correlation between the cytotoxic-like effects and the morphological transformation of the cells. This assumption is supported by the following observations: (a) vanadyl induces the weakest cytotoxic effect and also the weakest morphological transformation; (b) pervanadate causes a drastic decrease in the cell proliferation and strongly affects cellular morphology; and (c) 10 μM of any vanadium derivatives, a dose which stimulates cell growth, does not produce morphological transformations.

In BALB/3T3 cells, Sabbioni *et al*. (1991) studied the cytotoxicity and morphological transformations exerted by vanadium (V) and (IV). Whereas at 3–10 μM vanadyl did not transform cells, vanadate showed neoplastic transformation. In our Swiss 3T3 culture system, 2.5–10 μM vanadate stimulated cell growth. On the other hand, Sabbioni *et al*. (1991) also showed that vanadyl exerted a cytotoxic effect at doses at which no morphological transformations were induced. On the contrary, our studies show morphological but no proliferative changes at 50 μM vanadyl. Altogether, these observations suggest that the bioactivity of the vanadium compounds would depend, at least in part, on the cellular type used in the *in vitro* study.

Taking into account the vanadium oxidation state, the experimental data suggest that vanadium (V) compounds are more cytotoxic than vanadium (IV) at a concentration of 25 μM. Vanadates are usually pentacoordinated assuming a tetahedral structure but can convert to a trigonal bipyramid. Pervanadates show a coordination number of six, seven or eight, adopting the pentagonal bipyramid form (Stankiewicz & Tracey 1995). These features allow us to suggest that the coordination geometry may play a role in the deleterious effects of vanadium compounds in this cell line. Another possibility of the observed differences between the two vanadium (V) compounds tested could be due to a greater toxicity of the peroxo derivatives. This toxicity may be related to the ability to act as an oxidant of cellular thiols (Stankiewicz *et al*. 1995). Peroxovanadates are more potent oxidizing agents than vanadate, especially with regard to cysteine residues of some

enzymes. Such oxidations may led to irreversible enzyme inactivation. Hydrogen peroxide has been demonstrated to have similar insulin-mimetic effects to vanadate (Stankiewicz & Tracey 1995). However, the pervanadates show a significant enhancement of these effects. The mechanism of the toxic effect of vanadate is not currently known, even though inhibition of PTPases has been suggested (Lau *et al*. 1989). Using EPR spectroscopy, it has been shown that vanadate is reduced to vanadyl upon entry into cells (Degani *et al*. 1981, Heinz *et al*. 1982, Willsky & Dosch 1986, Sabbioni *et al*. 1993). By this biotransformation cells become protected from the toxic action of vanadate. The way in which cells might handle other vanadium derivatives, such as pervanadate, is still unknown and requires further investigation. Our results suggest that if the biotransformation mechanism is operative in fibroblasts, it is not efficient enough to prevent the toxicity of pervanadate. Thus, the higher toxicity of pervanadate as compared with vanadate could derive from the detoxifying pathway into the cells.

In brief, our results show that: (a) vanadium compounds induce mitogenic effects correlated with morphological transformations; (b) the morphological changes precede the proliferative alterations; and (c) the oxidation state and coordination geometry of the vanadium compounds could determine the magnitude of the cytotoxicity. This selectivity is likely to be related to the mechanism by which cells handle the metabolism of vanadium derivatives.

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## References

Bourgoin S, Grinstein S. 1992 Peroxides of vanadate induce activation of phospholipase D in HL-60 cells. Role of tyrosine phosphorylation. *J Biol Chem* **267**, 11908–11916.

Chen Y, Chan TM 1993 Orthovanadate and 2,3-dimethoxy-1,4-naphthoquinone augment growth factor-induced cell proliferation and c-*fos* gene expression in 3T3-L1 cells. *Arch Biochem Biophys* **305**, 9–16.

Cortizo AM, Etcheverry SB. 1995 Vanadium derivatives act as growth factor mimetic compounds upon differentiation and proliferation of osteoblast-like UMR106 cells. *Mol Cell Biochem* **145**, 97–102.

Davidai G, Lee A, Schuartz Y, Hazum E. 1992 PDGF induces tyrosinephosphorylation in osteoblast-like cells: relevance to mitogenesis. *Am J Physiol* **263**, E205–E209.

Degani H, Gochin M, Karlish JD, Shechter Y. 1981 Electron Paramagnetic Resonance Studies and Insulin-like Effects of Vanadium in Rat Adipocytes. *Biochemistry* **20**, 5795–5799.

Fantus G, Kadota S, Deragon G, Foster B, Posner B. 1989 Pervanadate [peroxide(s) of vanadate] mimics insulin action in rat adipocytes via activation of the insulin receptor tyrosine kinase. *Biochemistry* **28**, 8864–8871.

Gresser M, Tracey AS. 1990 Vanadates as phosphate analogs in biochemistry. In: Chasteen ND, ed. *Vanadium in Biological Systems*. The Netherlands: Kluwer Academic Publishers; 63–79.

Gresser M, Tracey AS, Stankiewicz P. 1987 The interaction of vanadate with tyrosine kinases and phosphatases. *Adv Prot Phosphatases* **4**, 35–57.

Heinz H, Rubinson KA, Grantham JJ. 1982 The transport and accumulation of oxyvanadium compounds in human erythrocytes *in vitro*. *J Lab Clin Med* **100**, 593–612.

Kadota S, Fantus IG, Deragon G, Guyda HJ, Posner BI. 1987 Stimulation of insulin-like growth factor II receptor binding and insulin receptor kinase activity in rat adipocytes. Effects of vanadate and $H_2O_2$. *J Biol Chem* **262**, 8252–8256.

Klarlund JK. 1985 Transformation of cells by an inhibitor of phosphatases acting on phosphotyrosine in proteins. *Cell* **41**, 707–711.

Lau KHW, Tanimoto H, Baylink D. 1988 Vanadate stimulates bone cell proliferation and bone collagen synthesis in vitro. *Endocrinology* **123**, 2858–2867.

Lau KHW, Farley J, Baylink D. 1989 Phosphotyrosyl protein phosphatases. *Biochem J* **257**, 23–36.

Leighton B, Cooper GJS, DaCosta C, Foot E. 1991 Peroxovanadates have full insulin-like effects on glycogen synthesis in normal and insulin-resistant skeletal muscle. *Biochem J* **276**, 289–292.

Lönnroth P, Eriksson JW, Posner BI, Smith U. 1993 Peroxovanadate but not vanadate exerts insulin-like effect in human adipocytes. *Diabetologia* **36**, 113–116.

Mountjoy KG, Flier JS. 1990 Vanadate regulates glucose transporter (Glut-1) expression in NIH3T3 mouse fibroblasts. *Endocrinology* **127**, 2025–2034.

Sabbioni E, Pozzi G, Pintar A, Casella L, Garattini S. 1991 Cellular retention, cytotoxicity and morphological transformation by vanadium(IV) and vanadium(V) in BALB/3T3 cell lines. *Carcinogenesis* **12**, 47–52.

Sabbioni E, Bonardi M, Gallorini M, *et al*. 1992 Application of radiotracers with high specific radioactivity to metallotoxicological studies. *J Radioanal Nucl Chem* **160**, 493–503.

Sabbioni E, Pozzi G, Devos S, *et al*. 1993 The intensity of vanadium(V)-induced cytotoxicity and morphological transformation in BALB/3T3 cells is dependent on glutathione-mediated bioreduction to vanadium(IV). *Carcinogenesis* **14**, 2565–2568.

Shechter Y. 1990 Insulin-mimetic effect of vanadate. Possible implications for future treatment of diabetes. *Diabetes* **39**, 1–5.

Shechter Y, Shisheva A. 1993 Vanadium salts and the future treatment of diabetes. *Endeavour* **17**, 27–31.

Shisheva A, Shechter Y. 1993 Mechanism of pervanadate stimulation and potentiation of insulin-activated glucose transport in rat adipocytes: dissociation from vanadate effect. *Endocrinology* **133**, 1562–1568.

Smith JB. 1983 Vanadium ions stimulate DNA synthesis in Swiss mouse 3T3 and 3T6 cells. *Proc Natl Acad Sci* ***80***, 6162–6166.

Stankiewicz PJ, Tracey AS. 1995 Stimulation of enzymes activity by oxovanadium complexes. In: Sigel H & Sigel A, eds. *Metal Ions in Biological Systems*. vol 31. *Vanadium and its Role in Life*. New York: Marcel Dekker, Inc.; 249–285.

Stankiewicz PJ, Tracey AS, Crans DC. 1995 Inhibition of phosphate-metabolizing enzymes by oxovanadium (V) complexes. In: Sigel H & Sigel A, eds. *Metal Ions in Biological Systems*. vol 31. *Vanadium and its Role in Life*. New York: Marcel Dekker, Inc.; 287–324.

Trudel S, Paquet MR, Grinstein S. 1991 Mechanism of vanadate-induced activation of tyrosine phosphorylation and of the respiratory burst in HL-60 cells. Role of reduced oxygen metabolites. *Biochem J* **276**, 611–619.

Wang H Scott RE. 1992 Induction of c-*jun* independent of PKC, pertussis toxin-sensitive G protein, and polyamines in quiescent SV40-transformed 3T3 T cells. *Exp Cell Res* **203**, 47–55.

Wang H, Scott RE. 1994 Distinct protein tyrosine phosphorylation during mitogenesis induced in quiescent SV40-transformed 3T3 T cells by insulin or vanadate. *J Cell Physiol* **158**, 408–416.

Willsky GR, Dosch S. 1986 Vanadium Metabolism in Wild Type and Respiratory-Deficient Strains of *S. cerevisiae*. *Yeast* **2**, 77–85.