casionally present on varnish, are rare (*2*). Although some fungi may incorporate atmospheric CO_2 into their biomass, the microcolonial fungi commonly found on varnish seem to obtain their carbon from airborne organic matter derived from adjacent soil and vegetation (*13*). Second, certain types of plants may contribute more than their share of organic debris to the atmosphere, thus leading to bias in varnish $\delta^{13}C$ values. Third, it is possible that varnish growth is favored during seasons when organic debris is shed by plants with nonrepresentative $\delta^{13}C$ values. Finally, $\delta^{13}C$ values of CAM and C_4 plants may change in response to variations in environmental factors (*19*). Fortunately, the isotopic changes these plants experience are such that the environmental signal in varnish organic matter $\delta^{13}C$ values would be enhanced, rather than weakened, by such changes.

In conclusion, our stable isotope analyses of organic matter in rock varnish reveal that $\delta^{13}C$ values are strongly correlated with environment and that $\delta^{13}C$ values in fossil varnish reflect past environmental conditions. The abundance of plants possessing different photosynthetic pathways in adjacent vegetation governs the carbon isotopic composition of rock varnish organic matter. However, before carbon isotope ratios of varnish organic matter can be used routinely as paleoenvironmental and paleoecological indicators, the method of extracting layers of fossil varnish only a few micrometers thick must be refined.

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5. Desert varnish deposits formed during the last 100 years consist of isolated patches only 1 micrometer thick. Since varnish contains less than 1 percent organic matter by weight, on the order of 10^5 cm^2 of varnish would have to be removed to provide enough organic matter for an isotopic determination. We had access to only a few historical varnished surfaces of this size in the western United States.
6. Varnish was removed from the underlying rock as described in (*3*). Temporal control was achieved by removing the entire varnish coat from landforms of Holocene age (for example, shorelines of western U.S. pluvial lakes) or by sampling only surficial varnish.
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8. $$\delta^{13}C = \left[\frac{(^{13}C/^{12}C_{sample})}{(^{13}C/^{12}C_{standard})} - 1\right] \times 1000$$
The standard is the PDB carbonate.
9. Organic matter was concentrated from varnish samples by removing manganese oxides with acidified hydroxylamine hydrochloride, iron oxides with dithionite, carbonates with HCl, and silicates with HF. Carbon isotopic composition was determined as described by D. W. Northfelt, M. J. DeNiro, and S. Epstein [*Geochim. Cosmochim. Acta* **42**, 495 (1981)].
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15. Microorganisms cultured from varnish have not been reported to be autotrophic (*11–13*). Isotopic fractionation occurring during heterotrophic growth of varnish microorganisms is probably small, since, in laboratory culture, the $\delta^{13}C$ values of the cell masses of heterotrophic (nonvarnish) microbes are typically within a few per mil of the $\delta^{13}C$ values of their carbohydrate carbon sources [P. H. Abelson and T. C. Hoering, *Proc. Natl. Acad. Sci. U.S.A.* **47**, 623 (1961); L. O. Ingram, J. A. Calder, C. Van Baalen, F. E. Plucker, P. L. Parker, *J. Bacteriol.* **114**, 695 (1973)].
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23. We thank L. Sternberg, S. Ambrose, A. Orme, and anonymous reviewers for comments, R. Gerson and J. Dohrenwend for field assistance, B. R. T. Simoneit for organic geochemical analyses of rock varnish, and H. Ajie and D. Winter for isotopic measurements. Supported in part by UCLA and NSF grant ATM 79-24591. This report is dedicated to Bernard Annenberg, who started the first author in the ways of science.

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Effect of Vanadate on Elevated Blood Glucose and Depressed Cardiac Performance of Diabetic Rats

Abstract. *The trace element vanadium has an unclear biological function. Vanadate, an oxidized form of vanadium, appears to have an insulin-like action. The effect of vanadate on blood glucose and cardiac performance was assessed in female Wistar rats 6 weeks after they were made diabetic with streptozotocin. When vanadate was administered for a 4-week period to the diabetic rats, their blood glucose was not significantly different from that of nondiabetic controls despite a low serum insulin. In contrast, blood glucose was increased about threefold in the diabetic rats that were not treated with vanadate; these rats also had low insulin levels. Cardiac performance was depressed in the untreated diabetic animals, but the cardiac performance of the vanadate-treated diabetic animals was not significantly different from that of nondiabetic controls. Thus vanadate controlled the high blood glucose and prevented the decline in cardiac performance due to diabetes.*

Vanadium, a trace element in the environment (*1, 2*), is found in plants and animals, but its biological function is unclear (*3*). Studies have shown that vanadium directly affects glucose metabolism (*1–7*). Tolman *et al.* (*1*) reported that vanadate stimulates glucose oxidation and transport in adipocytes, enhances glycogen synthesis in liver and diaphragm, and inhibits hepatic gluconeogenesis and intestinal glucose transport. An insulin-like effect of vanadate was also reported by other investigators (*2–7*). Vanadate can also influence cardiac performance. It induced a positive inotropic effect in the isolated rat heart (*8*) and isolated cat papillary muscle (*9*) and had a negative inotropic effect in most atria (*10*). These observations suggest that vanadate may have a role in regulating glucose metabolism and cardiac performance in vivo. We therefore investigated the possibility that vanadate can affect the elevated blood glucose concentration and depressed cardiac contraction of diabetic rats.

The animals used in this study are described in Table 1. Body weights of rats made diabetic with streptozotocin but not treated with vanadate were significantly lower than body weights of untreated controls. There was no significant difference between the weights of the two groups of vanadate-treated animals but they gained weight more slowly than did the controls; however, the dif-

ference was not significant. Growth retardation of vanadate-treated rats has been reported (*11*). The slow growth rate of vanadate-treated rats may be the result of an effect of this agent on cell division or cell growth, or both (*11*). Serum insulin levels were similar in the two groups of vanadate-treated animals and were slightly higher than those of the untreated diabetic group. These levels were, however, significantly lower in these three groups of animals than in the untreated controls. In contrast, blood glucose levels of the vanadate-treated rats were not significantly different from those of the controls. As expected, blood glucose increased nearly threefold in the animals made diabetic with streptozotocin but not treated with vanadate. These results strongly suggest that vanadate was involved in reducing the high serum glucose of the diabetic rats. An insulin-like effect of vanadate on glucose metabolism was observed in our preliminary studies (*12*) in which rats given an injection of streptozotocin did not appear to be diabetic as long as they consumed vanadate in their drinking fluid. Glycosuria, which was evident 1 to 3 days after vanadate was removed from the drinking fluid, disappeared within 24 hours when vanadate treatment was restored. Blood samples taken from the animals also showed normal serum glucose levels.

Since vanadate has an insulin-like effect on glucose metabolism, an increase in blood glucose might not trigger a concomitant increase in serum insulin as long as vanadate is present. This suggests that the insulin levels of the blood can decrease without affecting glucose levels, as long as vanadate is present. In the vanadate-treated controls, serum insulin was significantly depressed but serum glucose was not different from that in the untreated controls. This observation suggests that vanadate inhibited insulin secretion in the vanadate-treated control animals.

The mechanism by which vanadate decreased the high serum glucose of the vanadate-treated diabetic rats is a matter of speculation. Vanadate could have induced the effect by enhancing glucose transport across the cell membrane and subsequently stimulating glycogen synthesis or glycolysis. These effects of vanadate have been demonstrated by other investigators using adipocytes, liver cells, diaphragm (*1, 4*), and human erythrocytes (*5*). Whatever the mechanism, these results suggest that vanadate has an insulin-like effect on glucose metabolism in vivo.

Diabetes mellitus is frequently associated with myocardial dysfunction in humans even in the absence of associated coronary disease or hypertension (*13*). This study, like others (*13, 14*), confirmed this observation in that it revealed a depressed cardiac performance in the untreated diabetic rats (Fig. 1). In contrast, the performance of the hearts of the vanadate-treated diabetic rats was not significantly different from that of the untreated controls. The heart function of the vanadate-treated controls also appeared to be normal (Fig. 1). Hence, it seems that vanadate, like insulin (*14*), not only controlled the serum glucose levels of the vanadate-treated diabetic

Table 1. General features of the experimental rats. Female Wistar rats weighing 175 to 200 g were made diabetic with a single dose of streptozotocin (55 mg/kg, intravenously) dissolved in a citrate buffer (*p*H 4.5). Controls received the citrate injections only. Two weeks after diabetes induction, the rats were randomly divided into four groups: controls; vanadate-treated controls; streptozotocin-injected; and vanadate-treated, streptozotocin-injected. The nondiabetic controls and the streptozotocin-injected (diabetic) group drank a 0.5 percent NaCl solution. The vanadate-treated controls and the vanadate-treated diabetic group drank a 0.5 percent NaCl solution containing sodium orthovanadate (0.6 to 0.8 mg/ml). The animals were killed 6 weeks after diabetes induction. Diabetes was assessed periodically by tests for glycosuria (Lilly Test-Tape). Blood samples were collected from nonfasting animals at the time of death and assayed for glucose with a glucose kit (Sigma) and for insulin by standard radioimmunoassay techniques (Amersham kit).

Group	Rats (No.)	Body weight (g)	Plasma insulin (μU/ml)	Plasma glucose (mg/100 ml)
Control				
Untreated	6	230 ± 4	27.3 ± 4.2	143.0 ± 10.8
Vanadate-treated	8	215 ± 9	14.1 ± 2.8*	133.2 ± 4.5
Streptozotocin-injected				
Untreated	6	197 ± 6*	8.8 ± 0.9*	363.5 ± 1.6*†
Vanadate-treated	8	217 ± 6	12.4 ± 1.4*	157.7 ± 7.7

*Significantly different from untreated controls ($P < 0.05$). †Significantly different from vanadate-treated animals ($P < 0.05$) (*20*).

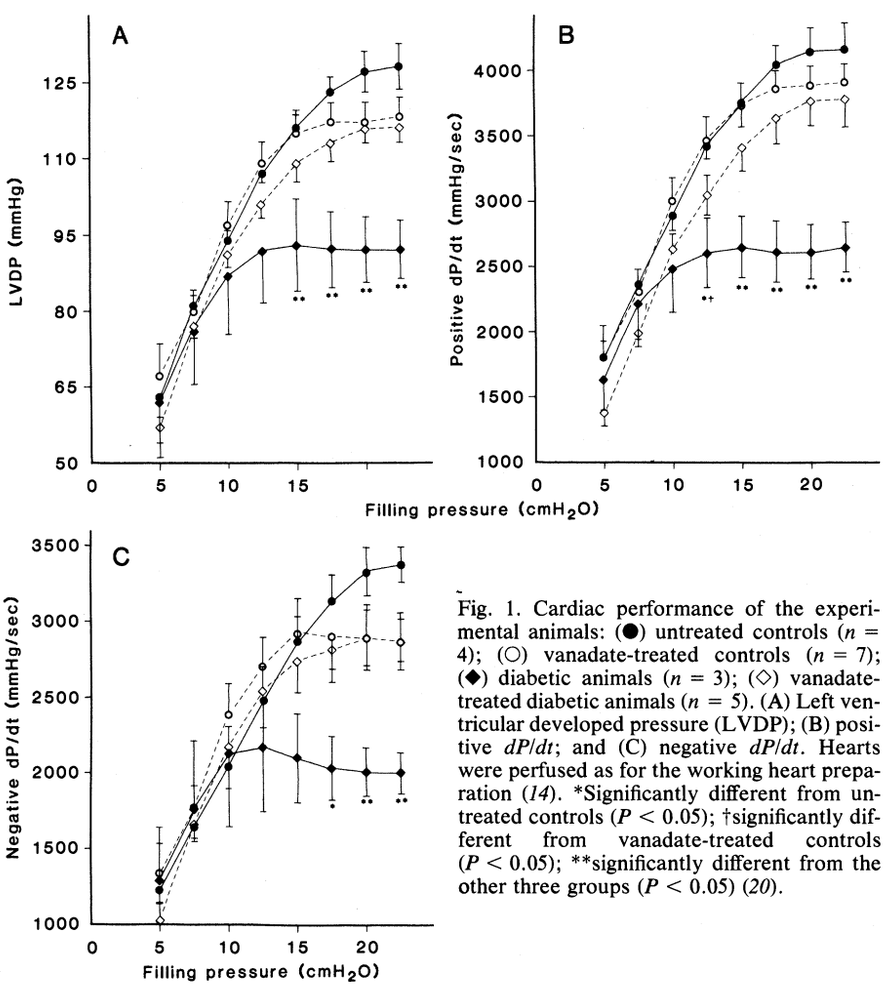

Fig. 1. Cardiac performance of the experimental animals: (●) untreated controls (n = 4); (○) vanadate-treated controls (n = 7); (◆) diabetic animals (n = 3); (◇) vanadate-treated diabetic animals (n = 5). (A) Left ventricular developed pressure (LVDP); (B) positive dP/dt; and (C) negative dP/dt. Hearts were perfused as for the working heart preparation (*14*). *Significantly different from untreated controls ($P < 0.05$); †significantly different from vanadate-treated controls ($P < 0.05$); **significantly different from the other three groups ($P < 0.05$) (*20*).

Table 2. Fluid and vanadate intake of experimental rats. Rats were given a fresh supply of drinking fluid every morning. Vanadate intake was determined as 1000 × [average fluid intake per day (milliliters) × concentration of vanadate (milligrams per milliliter)]/[body weight (grams)] (*21*).

Group	Rats (No.)	Fluid intake (ml/day)	Vanadate intake (mg/kg per day)	
			At 0.6 mg/ml	At 0.8 mg/ml
Control				
Untreated	6	108 ± 2*	0	0
Vanadate-treated	8	25 ± 1	71 ± 2	94 ± 2
Streptozotocin-injected				
Untreated	6	230 ± 5*†	0	0
Vanadate-treated	8	27 ± 1	75 ± 3	100 ± 3

*Significantly different from vanadate-treated animals ($P < 0.05$). †Significantly different from untreated controls ($P < 0.05$) (*20*).

rats but preserved their cardiac function as well.

Cardiac performance was not altered in the vanadate-treated controls. The reasons for this are not clear, but a possible explanation for this observation could be that a direct positive inotropic effect of vanadate, as seen in vitro, might have been offset in these animals by the cardiac response to lower insulin levels. Alternatively, vanadate may not exert a direct cardiotonic effect in vivo. In this study, there appears to be no evidence of a direct positive inotropic effect of vanadate on the heart.

The cellular mechanisms of action of vanadate on the heart are still not fully understood (*15*). It has been shown, for example, to inhibit myocardial sarcoplasmic reticular Ca^{2+}-dependent adenosinetriphosphatase (ATPase) (*16*); yet in our study the cardiac performance of the vanadate-treated animals was not significantly different from that of the controls. The reason for this apparent contradiction is still not clear. Perhaps when taken up by the cell, the inhibitory effect of this agent on organelle ATPases is drastically reduced or even eliminated. In fact, for vanadate to inhibit this ATPase, it must enter the cell, and sufficient quantities of cytoplasmic vanadate must remain, since vanadyl, the reduced form, is much less potent (*17*). Cytoplasmic conditions favor formation of the vanadyl form (*17*). Therefore, despite the inhibition of sarcoplasmic reticular Ca^{2+}-ATPase in vitro, inhibition of this ATPase may not be involved in the mechanism of action of vanadate in vivo. Another explanation could be that the concentration of vanadate that inhibited this enzyme in vitro was not reached in these animals. Similarly, sarcoplasmic reticular Ca^{2+}-ATPase is depressed in chronically diabetic rats (*18*), and yet we observed normal myocardial function in the vanadate-treated diabetic animals. Possible explanations for the cardiac effect of vanadate in the diabetic rats are: (i) the insulin-like effect of vanadate on myocardial metabolism prevented the mechanical changes observed in untreated diabetics; (ii) the fall in serum glucose led to the prevention of the myocardial alterations; and (iii) the positive inotropic effect of vanadate prevented the diabetic cardiac dysfunction. These explanations relate to possible mechanisms for the preservation of cardiac function in rats made diabetic for 6 weeks. The possibility that long-term diabetic cardiomyopathy can be reversed by vanadate requires investigation.

Rats that drank the vanadate solution consumed comparable amounts of the compound during the treatment period, but drank significantly less fluid than the animals that received only the NaCl solution. The diminished intake of fluid did not appear to have influenced the study since in a preliminary investigation streptozotocin-diabetic rats were still severely diabetic after drinking small volumes of water (Table 2). Further, these animals became severely dehydrated and weak within a 24-hour period. This phenomenon was not observed in any of the vanadate-treated rats used in this study. Vanadate ingestion might have induced water retention which subsequently was responsible for the diminished water intake. This possible effect of vanadate requires further investigation.

In addition, it does not appear that the presence of 0.5 percent NaCl in the drinking water had any influence on the results of this study, because the rats given streptozotocin but not treated with vanadate were still diabetic. In fact, the values we obtained for serum insulin and glucose concentrations and for contractile performance of the control and diabetic animals are similar to those reported elsewhere (*14*).

In conclusion, this study shows that vanadate can influence glucose metabolism and therefore may play a role in its regulation in vivo. It also suggests that vanadate can modify the cardiac abnormality frequently associated with diabetes mellitus.

While this paper was in preparation, Tamura *et al.* (*19*) reported that vanadate, like insulin, stimulated the phosphorylation of the 95,000-dalton subunit of the insulin receptor on the tyrosine residues, both in intact adipocytes and in a solubilized insulin receptor fraction. These results, which indicate that insulin and vanadate have similar initial actions on receptor phosphorylation and also act similarly on intracellular events such as the activation of glycogen synthase (*4, 19*) and glycolysis (*5*), support our findings of an apparent insulin-like effect of vanadate in the whole animal.

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levels of streptozotocin-diabetic rats was not fixed. In this study, 0.6 mg/ml prevented glycosuria in 75 percent of the animals, and 0.8 mg/ml was 100 percent successful in preventing glycosuria. The higher concentration was used beginning in the third week of treatment.
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Flow Effects on Prostacyclin Production by Cultured Human Endothelial Cells

Abstract. *Endothelial cell functions, such as arachidonic acid metabolism, may be modulated by membrane stresses induced by blood flow. The production of prostacyclin by primary human endothelial cell cultures subjected to pulsatile and steady flow shear stress was measured. The onset of flow led to a sudden increase in prostacyclin production, which decreased to a steady rate within several minutes. The steady-state production rate of cells subjected to pulsatile shear stress was more than twice that of cells exposed to steady shear stress and 16 times greater than that of cells in stationary culture.*

Hemodynamic shear stresses have an important role in both the normal physiology and the pathobiology of the vascular endothelium. Morphologic and cytoskeletal changes occur in cultured endothelial cells subjected to shear stress (*1*) that closely mimic those in vivo (*2*). Furthermore, functional responses to shear stress have also been reported, such as increases in histamine-forming capacity (*3*) and transient increases in fluid endocytosis of cultured endothelial cells in response to step changes in shear stress (*4*). One feature of the endothelium that appears to be readily influenced by shear stress is the production of prostacyclin (PGI_2) (*5, 6*). PGI_2 is a potent vasodilator and the most potent endogenous inhibitor of platelet aggregation known (*7*); thus, it may contribute to the nonthrombogenicity of the endothelial lining of the vessel wall and to the regulation of local blood flow. Because PGI_2 has a short half-life (3 minutes) and is produced in low amounts by unstimulated cultured endothelial cells (*8, 9*), there is controversy over the concentrations of circulating PGI_2 that exist under physiologic flow conditions in vivo (*10*). We now describe our study of the production rates of PGI_2 in vitro by primary cultured human endothelial cells subjected to steady and pulsatile shear stresses.

To simulate the hemodynamic environment of the circulation and to examine the effect of various fluid shear stresses on the endothelium, we have developed a flow apparatus to subject cultured cells (*11*) to well-characterized shear (Fig. 1). The apparatus consists of two reservoirs situated one above the other with a parallel-plate flow chamber positioned between them. The flow through the chamber is driven by the hydrostatic pressure head created by the vertical distance between the two reservoirs (*12*). Pulsatile flow is introduced by a cam-driven clamp flow oscillator near the chamber. Monolayers of endothelial cells are subjected to a mean shear stress of 10 dyne/cm^2 for 7 to 8 hours. The flow oscillator generates a pulsatile flow that produces maximum and minimum shear stresses of 12 and 8 dyne/cm^2, respectively, at a frequency of 1 Hz. The resulting waveform approximates a square wave. The shear stresses are comparable to those estimated to be present in the circulation (*13*).

During an experiment, samples of circulating medium were drawn every 5 to 30 minutes, and the flow circuit was simultaneously replenished with fresh medium to maintain a constant circulating volume of 20 ml. Radioimmunoassay for 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, was performed on each sample in triplicate (*14, 15*). These concentrations were used to calculate the cumulative production of PGI_2 with respect to time (*15*). At the end of each experiment, cells were photographed and counted.

The onset of flow led to a sudden increase in production of PGI_2, which decreased to a constant rate within several minutes. By means of a Marquardt nonlinear regression analysis, measure-

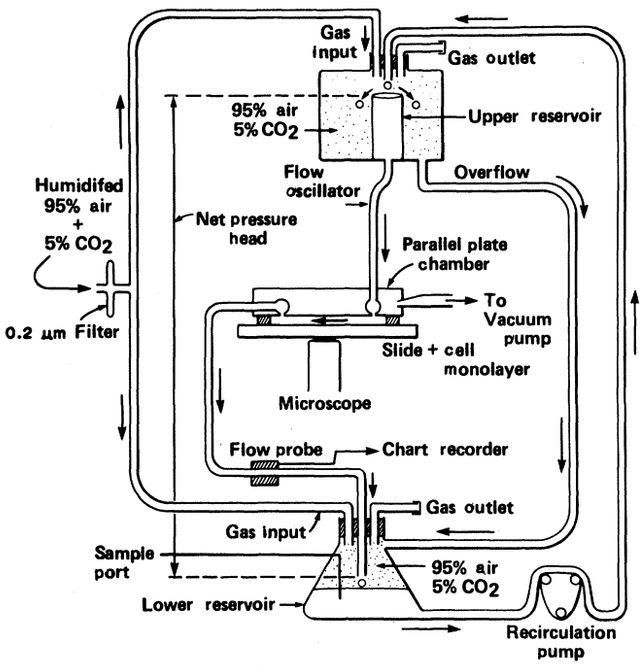

Fig. 1. Schematic diagram of the flow chamber, showing the polycarbonate plate, the rectangular Silastic gasket, and the glass slide with the attached endothelial monolayer. These are held together by a vacuum maintained at the periphery of the slide, forming a channel of parallel-plate geometry. The polycarbonate plate has two slits through which medium enters and exits the channel. The flow rate is controlled by either adjusting the relative distance between the two reservoirs or via a Harvard hemodialysis clamp upstream of the chamber. The flow rate is monitored by an electromagnetic flow probe. The medium is recirculated from the lower reservoir to the upper reservoir by a roller pump. The channel depth was 220 µm, and the area of cells exposed to flow was 16 cm^2. The entire circuit was kept at 37°C by an air curtain incubator. The *p*H was maintained at physiologic levels by gassing the medium with a humidified mixture of 95 percent air and 5 percent CO_2. Medium samples were taken from the lower reservoir, thereby avoiding any disturbance of the flow field. The medium residence time in the flow chamber and its tubing for the experiments was 15 seconds.