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-

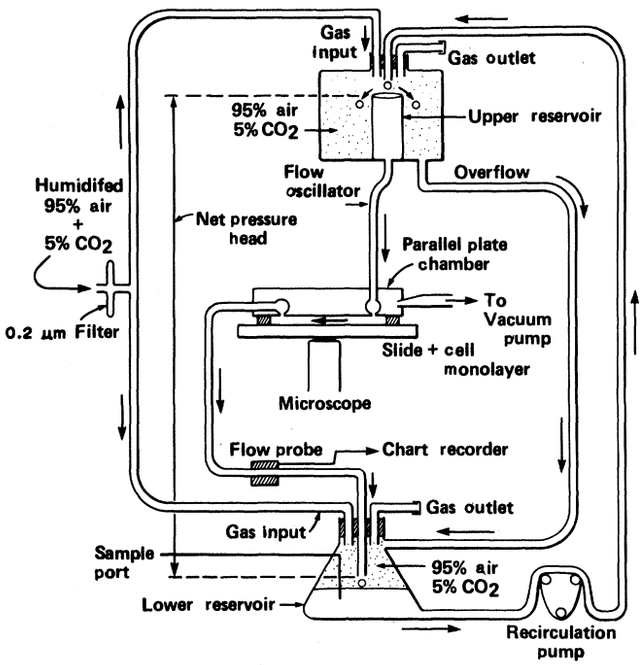


Fig. 1. Schematic diagram of the flow chamber, showing the polycarbonate plate, the rectangular Silastic gasket, and the glass slide with the attached endothelial monolayer. These are held together by a vacuum maintained at the periphery of the slide, forming a channel of parallel-plate geometry. The 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.

Table 1. Rates of PGI_2 production (picograms per 10^6 cells per minute) by replicate cultures of endothelial cells subjected to pulsatile, steady, and near-zero shear stress. Pulsatile shear stress had a square waveform (frequency, 1 Hz; range, 12 to 8 dyne/cm^2). Steady and near-zero shear stresses were 10 and 0.016 dyne/cm^2, respectively. Values are the mean of three experiments (± S.E.M.). The steady-state production rate of PGI_2 was given by parameter D, the peak production rate by $BC + D$, and the production decay constant by C^{-1} (Eq. 2).

Shear stress	Production rate		Decay constant (minutes)
	Steady state	Peak	
Pulsatile	161 ± 56	412 ± 155	7.4 ± 5.6
Steady	66 ± 18	368 ± 120	25.8 ± 10.4
(Ratio)*	(2.4)	(1.1)	(0.28)
Near-zero	11 ± 0.5		

*Ratio of pulsatile and steady shear stress values.

Table 2. Comparison of the steady-state production rates of PGI_2 (picograms per 10^6 cells per minute) induced by pulsatile and steady shear stress in the same monolayer of endothelial cells.

Experiment	Shear stress		Ratio*
	Pulsatile	Steady	
1	167	80	
2	55	22	
3	283	93	
Mean	168	66	2.5
S.E.M.	64	16	

*Ratio of pulsatile and steady shear stress values.

ments of cumulative production of PGI_2 were fitted to a four-parameter equation (Eq. 1),

$$P_c = A + B\,[1 - \exp(-CT)] + DT \quad (1)$$

where P_c is the cumulative production of PGI_2, T is time, and A, B, C, and D are the parameters to be determined. By taking the time derivative of Eq. 1, a smooth expression for the rate of production (P) as a function of time is obtained (Eq. 2).

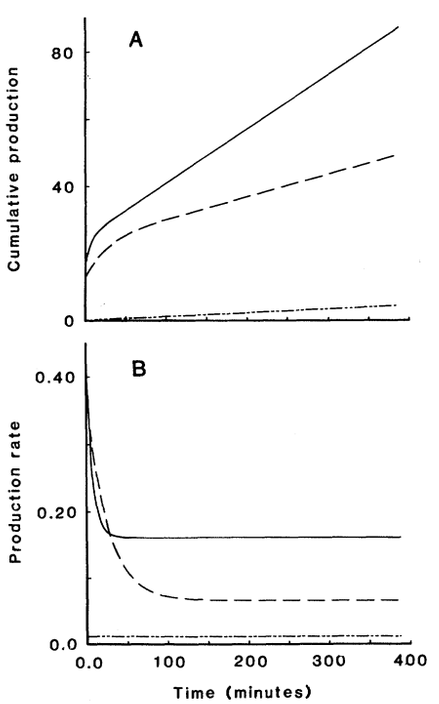
$$P = BC \exp(-CT) + D \quad (2)$$

The parameters calculated by the regression analysis can be related to the kinetics of the rate of production. The parameter D represents the steady-state or long-term production rate (SSR), and C is a measure of the rapidity of decay of the initial transient burst in production of PGI_2. Furthermore, the peak production rate can be expressed as the rate of production at time zero ($BC + D$).

The SSR of endothelial cells subjected to steady flow was 61 ± 8 pg of PGI_2 per 10^6 cells per minute [mean ± standard error of the mean (S.E.M.); $n = 11$], and cells subjected to pulsatile flow produced 136 ± 26 pg per 10^6 cells per minute ($n = 7$). The SSR of cells exposed to pulsatile shear stress was thus 2.2 times greater than that for cells exposed to steady shear stress (two-sided Wilcoxon test; $P < 0.005$). To eliminate variations due to individual donors and monolayer age, we ran a concurrent series of dual flow-loop experiments. Identical flow loops were assembled, in which one monolayer was subjected to steady flow and the other to pulsatile flow. Replicate cell cultures from the same umbilical cord were used, and sampling was done simultaneously (Fig. 2). The ratio of the mean SSR of endothelial cells subjected to pulsatile flow versus that from cells exposed to the same average shear stress under steady flow was 2.4 (Table 1). The ratio of peak production rates (Table 1) of pulsatile versus steady shear stress was not significantly different from unity (1.1). Therefore, the peak production rates for steady and pulsatile shear stress from equal mean flow were essentially the same. For comparison of the effects of steady and pulsatile shear stress on the same cell monolayer, cells were first exposed to steady flow for 2 hours and then to pulsatile flow for 4 hours (Fig. 3). Pulsatile flow was introduced by turning on the flow oscillator without stopping the flow. The ratio of the SSR during pulsatile flow to that during steady flow was 2.5 (Table 2).

The rate of basal release of PGI_2 from human umbilical vein endothelial cells under quiescent culture conditions was 10 pg per 10^6 cells per minute. In experiments designed to control the conditions in the flow chamber, cell monolayers in the chamber were perfused at a near-zero shear stress (0.016 dyne/cm^2) and produced 11 ± 0.5 pg of PGI_2 per 10^6 cells per minute.

Comparison of the light micrographs of the cell cultures before and after each experiment revealed no significant changes in cell number or morphology. Furthermore, continuous video microscopy and time-lapse recording during experiments did not indicate any visible cellular alterations during flow for these relatively short exposure times. Ibuprofen (250 μM) was used to inhibit cyclooxygenation of arachidonic acid and prevented PGI_2 production induced by either steady or pulsatile flow.



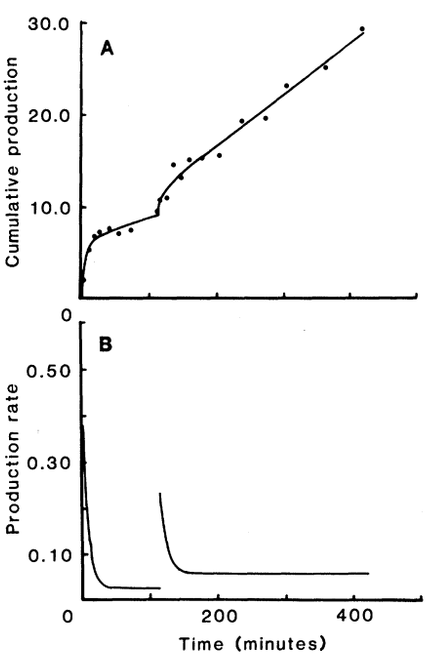


Fig. 2 (left). (A) The composite cumulative production profiles (nanograms of PGI_2 per 10^6 cells) of replicate cultures subjected to pulsatile (——), steady (– – –), and near-zero (– · · –) flow. The curves were constructed from the averaged parameters given in Table 1. (B) The composite rate of production of PGI_2 (nanograms per 10^6 cells per minute) by cells subjected to pulsatile, steady, and near-zero flow. Fig. 3 (right). (A) The cumulative production of PGI_2 (nanograms per 10^6 cells) by cells subjected for 2 hours to steady flow and then to pulsatile flow (see experiment 2 in Table 2). (B) The rate of production of PGI_2 (nanograms per 10^6 cells per minute).

A possible interpretation of the profiles for the production rates is that the onset (or the cessation) of flow and its subsequent continuation stimulate the cells by two independent mechanisms. This is best seen qualitatively in the expression for the production rate (Eq. 2). The first term represents a transient decay in production after the initial perturbation, with the rapidity of decrease determined by the time constant C^{-1}. The step change in shear stress at the onset of flow may represent an acute stimulation in which cell response attenuates within several minutes. The second term (D) represents a constant production rate that dominates at long times (large values of CT). The continuation of flow provides a continuous stimulus, the degree of which is a function of the qualitative nature and possibly the magnitude of the flow. This may explain why the peak production rates for the two flow profiles with identical mean shear stresses were the same yet led to different steady-state rates.

Using a nonrecirculating flow system, Grabowski *et al.* (*5*) found that cultured bovine aortic endothelial cells subjected to a step change in steady shear stress responded with a burst in the PGI_2 production rate that decayed within minutes. Furthermore, the peak production rate was a function of the magnitude of the step change in the shear stress (*5*). These findings are consistent with our data.

Our results suggest that production of PGI_2 by the vascular endothelium under physiologic flow conditions may be significantly higher than that reported in studies based on cells cultured under stationary conditions (*9*). Furthermore, the lower concentrations of PGI_2 reported for veins relative to arteries may be due to the relative lack of pulsatile flow and the lower shear stress in veins. Our findings provide further evidence that the qualitative nature of blood flow may have a controlling role in endothelial cell function.

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11. Human umbilical vein endothelial cells were harvested from umbilical cords by means of culture procedures adapted from M. A. Gimbrone, Jr. [*Prog. Hemostasis Thromb.* **3**, 1 (1976)]. For removal of the endothelial cells, the veins were cannulated, rinsed with 100 ml of phosphate-buffered saline (PBS), filled with 0.03 percent collagenase in medium 199 (Gibco), and incubated for 30 minutes at room temperature. After incubation, the enzyme solution was flushed through the cord with 100 ml of PBS, and the effluent was collected and centrifuged at 100*g* for 10 minutes. The cell pellet was resuspended in medium 199 supplemented with 20 percent fetal bovine serum (Hyclone), 100 units of penicillin per milliliter, and 100 mg of streptomycin per milliliter. The cell suspension was seeded onto glass slides (75 by 38 mm; Fisher) that had been treated earlier with 0.5*M* NaOH for 2 to 3 hours and rinsed. Two slides were seeded per cord (5.0×10^4 to 1.0×10^5 cells per slide). Cultures became confluent after 3 or 4 days, and experiments were run 3 days after the cultures reached confluency.
12. For each experiment, the flow circuit was assembled and was autoclave sterilized. Under a laminar flow hood, the circuit was primed with 20 ml of culture medium, and a slide with a monolayer of cultured cells was positioned to form one of the plates of the parallel-plate chamber. Care was taken to avoid entrapment of air bubbles in the flow channel. The flow apparatus was then taken to the 37°C air curtain, where the gassing and roller pump connections were made.
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14. Samples were drawn into ibuprofen (Sigma) solution to give a final concentration of 1 m*M* to inhibit PGI_2 production by any suspended cells. Sample and replenishing volumes were both 1 ml. Attached cells were suspended by trypsinization and counted with a hemocytometer to determine the total viable count, as determined by trypan-blue exclusion staining.
15. PGI_2 was assayed by radioimmunoassay of the nonenzymatic breakdown product 6-keto-$PGF_{1\alpha}$. Before assaying, the samples were incubated at 37°C for 1/2 to 1 hour. Antibody to 6-keto-$PGF_{1\alpha}$ was obtained from Seragen. The assay sensitivity was 5 pg per 0.1 ml, and the cross-reactivity of the antibody with other culture media components was negligible. Sample concentrations were computed from scintillation counter data with a computer program modified from D. Rodbard *et al.* [*Radioimmunoassay* **1**, 469 (1978)]. Cumulative production of PGI_2 was determined by performing a mass balance over the flow loop, taking into account samples withdrawn and medium replenished.
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Xylem-Tapping Mistletoes: Water or Nutrient Parasites?

Abstract. *Most mistletoes parasitize higher plants by tapping the xylem (a conduction tissue) of their hosts. Field observations of diurnal gas exchange parameters and carbon isotope ratios in xylem-tapping mistletoes from three continents support the hypotheses that water use efficiency and carbon isotope composition are related and that mistletoes which are parasitic for water are also nutrient parasites, differing in their water use efficiency relative to that of their hosts on the basis of host nitrogen supply in the transpiration stream.*

Mistletoes are obligate epiphytic parasites of higher plant species (*1*). While a few mistletoes derive substantial nutritional benefits by connecting directly to the host's phloem tissues, most mistletoes tap only the xylem tissues and derive no nutritional benefit from their hosts other than the small amounts of organic carbon and nutrients carried in the transpiration stream (*1–3*).

While much is known about the anatomy and systematics of xylem-tapping mistletoes (*1, 3, 4*), less is known about their carbon, water, and nutrient relations (*3*), and several unusual physiological features of mistletoes have not been adequately explained (*5*). One such feature is that mistletoe leaves tend to have transpiration rates several times higher than those of their hosts (*1, 3, 5, 6*). Another is that they accumulate large amounts of calcium, potassium, and phosphorus and smaller amounts of nitrogen (*1, 3, 5, 7*). This holds true for autoparasitic mistletoes as well (*8*). Because xylem-tapping mistletoes exhibit high species diversity in arid and semiarid regions of the world (*1*), it is possible that this type of parasitism evolved principally as a means of water acquisition in water-limited habitats. However, many of these regions are also extremely nutrient-poor, and xylem-tapping parasitism may have evolved principally as a means of nutrient acquisition.

In this report we provide evidence, from many xylem-tapping mistletoe species at different global locations, that supports the hypothesis of Schulze *et al.* (*5*), namely that the unusually high transpiration rates of these mistletoes are in part a mechanism to acquire nitrogen, the nutrient potentially most limiting to their growth. To do this we compared