tonium; all three of these actinides were least soluble in this water.

Because all actinides in the same oxidation state display similar chemical behavior, the americium results may be assumed to be generally applicable to curium. Application to Pu(III), however, is less certain because of the ease with which this element can be converted to higher oxidation states with different chemical properties.

Because the primary purpose of this study was to compare the abilities of various ground waters to maintain neptunium and americium in solution, we made no attempt to use equal concentrations of the two elements. However, we also compared the behavior of each element in the ground waters, using americium and plutonium concentrations comparable to that of neptunium ($10^{-8}M$). We used only basalt and shale ground waters and sampled the solutions after 17 days at 90°C. The results (Table 5) are very similar to those obtained at lower concentrations and suggest that the reported solubilities are not saturation-limited but are valid over a range of concentrations. All species except Am(III) exhibited similar—and almost complete—solubility in basalt ground water, whereas all were essentially insoluble in shale ground water. The behavior of neptunium was somewhat different at 25°C.

We conclude that mobilization of all four actinides in these ground waters was minimized by reducing conditions and by high concentrations of sulfate ion, but it is uncertain whether the effect of sulfate could prevail in all ground waters. In contrast to the behavior observed for plutonium, the presence of high concentrations of free fluoride had no discernible effect on neptunium and americium solubilities. These characteristics vary with location within any given host rock type, and hence they are site-specific. Because of this, we propose that the chemical composition of the associated ground water and actual speciation experiments be included along with other relevant parameters as criteria in the selection of geologic sites as possible repositories for nuclear waste.

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## Inhibitory Role of the Endothelium in the Response of Isolated Coronary Arteries to Platelets

Abstract. *Aggregating autologous platelets caused contraction of isolated rings of canine left circumflex arteries. The contractions were augmented after removal of the endothelium and were attenuated by serotonergic antagonists. During contraction caused by prostaglandin $F_{2\alpha}$, aggregating platelets caused a transient increase in tension followed by a profound relaxation of arteries with endothelium, but caused only further contraction of arteries without endothelium. These observations demonstrate the importance of the vascular endothelium in opposing the constriction of coronary vessels caused by 5-hydroxytryptamine and other substances released from aggregating platelets.*

When platelets aggregate, they release a number of substances including 5-hydroxytryptamine (5HT) and thromboxane $A_2$, both of which can cause contraction of vascular smooth muscle cells (*1*).

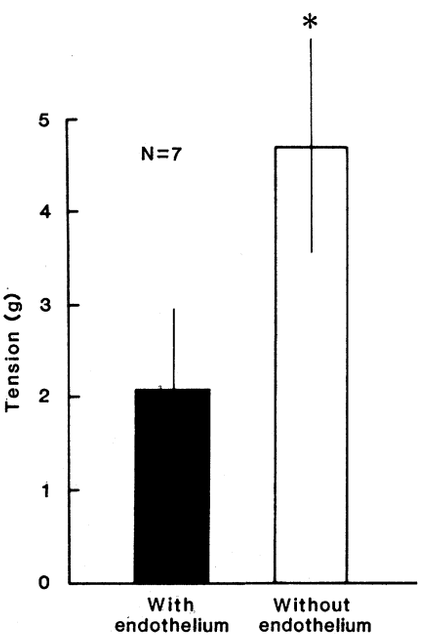


Fig. 1. Influence of endothelium on maximum contractile response of left circumflex artery rings to aggregating platelets. Addition of platelet suspensions resulted in contractions that reached a maximum within 10 minutes. The asterisk indicates a significant difference ($P < .05$) by Student's *t*-test for paired comparisons between arterial rings from the same vessel with and without endothelium. There was no significant difference in the contractions of rings with or without endothelium to $4 \times 10^{-2}M$ potassium chloride (11 ± 0.9 and 11 ± 1.0 g, respectively). To exclude α- or β-adrenergic and cholinergic actions all experiments were performed in the presence of phentolamine, propranolol, and atropine ($10^{-6}M$).

The endothelial cells play a major protective role by producing prostacyclin which inhibits platelet aggregation (*2*). The permeation of 5HT released from platelets to the smooth muscle cells may be limited by its enzymatic destruction by the monoamine oxidase in the endothelial cells (*3*). In addition, arterial endothelial cells can respond to a variety of agents by causing inhibition of the contractile process of the vascular smooth muscle (*4, 5*). The present study was designed to determine the role of the endothelium in the response of coronary vascular smooth muscle to aggregating platelets and to determine to what extent 5HT released by the platelets can be held responsible for the observed responses.

The left circumflex artery was dissected from the heart of mongrel dogs. Paired segments of vessel, 4 mm in length, were cut; in one the endothelium was removed by gentle rubbing of the intimal surface (*4, 5*). Rings were suspended in organ chambers filled with physiological salt solution (4 ml), and isometric tension was measured after equilibration of the rings at the optimal resting tension for contraction (*6*). In each study the integrity of the endothelium was demonstrated by the concentration-dependent relaxations induced by acetylcholine during contraction with prostaglandin $F_{2\alpha}$; no significant relaxation occurred in vessels denuded of endothelium (*7*). Autologous blood was collected in an anticoagulant consisting of acid, citrate, and dextrose. Platelet rich plasma was obtained by centrifugation (180*g* for 10 minutes). A pellet of platelets was obtained (1600*g* for 10 minutes) and resuspended in calcium-free saline containing 0.4 percent citrate at

*p*H 6.5 (*8*). Portions of this platelet suspension (100 μl) were added to the organ chamber containing the isolated arteries resulting in concentrations of 6.2 ± 1.0 × $10^7$ platelets per milliliter ($N$ = 12). An equal volume of supernatant, prepared by centrifugation (1600*g* for 10 minutes) of platelet suspensions, when added to the organ chamber had no significant effects on vessel tension. Spontaneous aggregation followed addition of the platelet suspensions to the physiological salt solution which contained 2.5 m*M* calcium. Aggregation was evidenced by gradual clearing of the initially turbid solution, visible platelet clumping, and platelet aggregates on the blood vessel surface observed with scanning electron microscopy. Under identical experimental conditions to those of the present study, 5HT and thromboxane $B_2$ were found in the supernatant (1600*g* for 10 minutes) of the physiological salt solution following platelet aggregation.

In the endothelium-denuded rings the contraction induced by the platelets was significantly larger than in those in which the intima was left intact (Fig. 1). The serotonergic antagonists ketanserin and cyproheptadine (*9*) significantly reduced contractions evoked by the platelets in endothelium-denuded rings, indicating that 5HT contributes to the response (*10*). The smaller contraction in the endothelium-containing preparations cannot be attributed to breakdown of 5HT by endothelial monoamine oxidase since the difference between rings with and without endothelium was not affected by the monoamine oxidase inhibitors Lilly-51641 ($10^{-5}M$) and semicarbazide ($10^{-5}M$) (*3, 11*).

These observations suggest that endothelial cells, when exposed to aggregating platelets, can generate an inhibitory signal affecting the underlying smooth muscle cells. Additional evidence for this interpretation is provided by studies on coronary arteries contracted with prostaglandin $F_{2\alpha}$, as shown in Fig. 2. During such contraction, the addition of platelet suspensions to the organ chambers caused significant relaxations in the rings with endothelium (from 4.9 ± 0.3 to 0.7 ± 0.2 g; $N$ = 6; $P < .05$); the relaxation was preceded by a moderate increase in tension. In two of six studies, the relaxation was prevented by prior treatment of the coronary ring with methysergide ($10^{-7}M$); prior treatment with ketanserin ($10^{-6}M$) had no effect. In the endothelium-denuded preparations the only significant effect of platelets was to cause a further increase in tension (from 5.1 ± 0.7 to 6.9 ± 0.7 g; $P < .05$; Fig. 2).

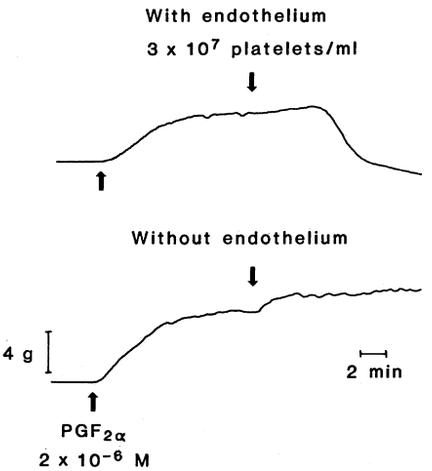


Fig. 2. Examples of the response of left circumflex artery rings with and without endothelium precontracted by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) upon addition of platelet suspensions. Similar responses to platelets were observed in arteries from six animals studied consecutively. The size of the contraction in response to $PGF_{2\alpha}$ was not significantly different in rings with or without endothelium (4.9 ± 0.3 and 5.1 ± 0.7 g, respectively; $N$ = 6).

It is apparent that the response to platelets is the net result of a direct contractile action on coronary smooth muscle and an indirect inhibitory action mediated by the endothelium. The initiator of the inhibitory responses has not been determined, but it is likely to result from the action on the endothelium of substances, including 5HT, released from the aggregating platelets. However, the failure of serotonergic antagonists to consistently block the platelet-induced relaxation make it unlikely that 5HT is the only factor involved in the endothelium-dependent inhibitory response evoked by platelets. It may be that other substances released by platelets participate in stimulating the inhibitory action of the endothelium. For example, adenosine diphosphate, which is released by platelets, is known to cause endothelium-dependent arterial relaxations (*5*).

The inhibition of cyclooxygenase with indomethacin ($10^{-5}M$), prostacyclin synthetase with tranylcypromine ($10^{-5}M$), or lipoxygenase with nordihydroguiaretic acid ($5 \times 10^{-5}M$) failed to block the relaxation response to aggregating platelets, indicating that synthesis of arachidonic acid metabolites by the endothelium, in particular prostacyclin, is probably not involved (*12, 13*).

The present study illustrates a protective role of the endothelium in curtailing vasoconstrictor episodes elicited by substances released from aggregating platelets. It also suggests a physiological role for endothelium-mediated relaxations of the smooth muscle, which so far have been mainly of pharmacological interest (*4, 5*). These mechanisms may be particularly pertinent to the pathophysiology of episodes of coronary vasospasm which may be initiated at sites of intimal damage (*14*). The absence of endothelium would permit full expression of the contractile actions of substances released from platelets aggregating at such a site. The demonstration that platelets evoke relaxation of coronary arteries if the endothelial cells are present may imply that if platelet aggregation were initiated at a point of blood vessel damage endothelial cells in the vicinity might trigger dilatation, favoring the removal of the platelet aggregates.

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