high-frequency solution. Any theoretical curve calculated by assuming an $\alpha_c > 1$ (and $Re_c = 30$) would lie between the two curves for $Re_c = 30$, $\alpha_c = 1$ and $Re_c = 30$, $\alpha_c = \infty$.

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Vascular Permeation of Platelet Factor 4 After Endothelial Injury

Abstract. *Antibody to platelet factor 4 was used to demonstrate permeation of this factor into the blood vessel wall after endothelial injury in rabbits. The presence of platelet factor 4 antigen in the vessel wall after removal of the endothelium was shown by immunofluorescence 10 and 30 minutes after injury but not 240 minutes afterward. This study demonstrates that factors carried by platelets can enter the vessel wall and that the movement of these platelet products into the vasculature is a short-lived, self-limiting process.*

After vascular injury, platelets adhere to exposed subendothelium, degranulate, and form aggregate masses (*1*). Following activation, they secrete numerous biochemical mediators including lysosomal enzymes, a potent vasoconstrictor (thromboxane A_2), and a mitogenic protein [platelet-derived growth factor (PDGF)] that stimulates growth of cultured smooth muscle cells. Studies of vascular injury have focused on the morphology of platelet adhesion to the subendothelium, the ensuing aggregation (*2*), and platelet turnover kinetics (*3*). However, until now there was no direct evidence that materials secreted by the platelet enter the vessel wall. To establish that such materials do enter the vessel wall and to follow their course in vivo, we studied a well-known platelet-specific alpha-granule protein, platelet factor 4 (PF4), which is a 7800-dalton polypeptide that can neutralize heparin's anticoagulant effect and may interact with other sulfated glycosaminoglycans (*4*, *5*). In our study, the indirect method of immunofluorescence was used to demonstrate (i) the presence of PF4 antigen in the vessel wall within minutes of platelet attachment to the site of endothelial injury and (ii) the disappearance of the antigen after 4 hours.

We isolated PF4 protein from rabbit platelets (*5*) and then raised in sheep a monospecific antibody to the purified protein (*6*). Rabbit iliac arteries were denuded of endothelium with a low-pressure balloon catheter (*1*). At selected times after injury, the animals were killed by exsanguination and sections of iliac artery were prepared for testing with indirect immunofluorescence techniques adapted for the PF4 antigen (*7*).

The PF4 antigen was identified at the luminal surface and in the tunica media of the artery 10 and 30 minutes after removal of the endothelium (Fig. 1). Immunofluorescent platelets were seen on the vessel surface 240 minutes after injury, although there was a marked diminution in immunofluorescent staining of the vessel wall. These results are consistent with those of morphological studies (*1*) and provide additional information about the reaction between platelets and the vessel wall several hours after injury. They also agree with the results of recent studies of ^{51}Cr-labeled platelet turnover (*3*). In those studies, platelet interaction with the vessel wall was evaluated in rabbits whose endothelium was removed with a balloon catheter. It was shown that only 0.2 percent of the circulating platelets attached to the damaged wall and that turnover was almost undetectable. Similarly, we show here that secretion of antigen by platelets is a short-lived, self-limiting phenomenon, with apparently little secretion occurring 4 hours after injury.

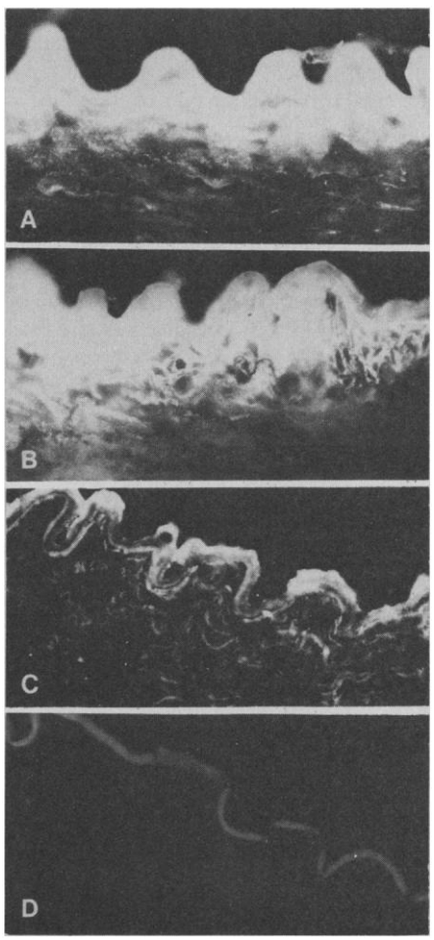

Fig. 1. Localization of PF4 in the vessel wall with sheep antibody to rabbit PF4 at (A) 10, (B) 30, and (C) 240 minutes after endothelial injury (×250). Note permeation of PF4 into the vessel wall after 10 and 30 minutes, and its exclusive presence in the luminal platelets after 240 minutes. (D) Control slide incubated with antigen-absorbed antibody; immunofluorescent staining did not occur (×250). Slides containing hyperimmune sheep serums incubated under the same conditions also did not show immunofluorescence.

It has been well documented that the subendothelium is covered by platelets within minutes of endothelial injury. After 10 minutes, the platelets spread out to completely cover the exposed surface (*1*). Platelets adhering to the subendothelial surface lose 97 percent of their alpha granules after 40 minutes, whereas most of the platelets that aggregate onto the adherent platelets retain their granules (*2*). Our results demonstrate, apparently for the first time, the permeation of a platelet-derived protein into the vessel wall: the platelet-derived antigen appears in the vessel wall within minutes of removal of the endothelium.

Since PF4 and other platelet-secreted proteins like PDGF reside in the same granule population and are secreted in response to the same stimuli (*8*), it seems likely that these proteins also enter the vessel wall after removal of the endothelium. Recent studies suggest that only a transient exposure to PDGF is needed to cause proliferation of cultured cells (*9*, *10*). Previous studies in our laboratory showed that the proliferative response of vascular smooth muscle cells (SMC) is self-limiting, with peak SMC synthesis of DNA occurring after 48 hours, followed by a dramatic decrease (*10*). These studies suggest that the rapid increase in secretion by platelets after injury is fol-

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lowed by a self-limiting proliferative response. Factors limiting this platelet reactivity and SMC proliferation are unknown.

The direct demonstration of movement of a platelet-specific protein into the vessel wall provides a new tool for studying platelet-vessel wall interactions. Since platelets contain and secrete a variety of other biologically active materials including lysosomal enzymes and a heparin-cleaving enzyme, these materials may also enter the vessel wall and could influence vascular repair. The ability to identify and trace the movement of one platelet protein into the vessel wall should facilitate further study of platelet products in the circulatory system.

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6. Antiserum (100-μl portions) was mixed with equal volumes of buffer containing between 10 and 100 μg of PF4 protein, incubated at 4°C for 72 hours, and centrifuged at 10,000*g*. The precipitate was washed with 150 m*M* NaCl and 10 m*M* phosphate-buffered saline (PBS) (*p*H 7.6), and the proteins in the precipitate were quantified with the Lowry technique. The absorbed antiserum from the equivalence zone of the precipitin curve was analyzed for residual immunoreactivity with the Ouchterlony immunodiffusion technique. Preimmunization, postimmunization, and absorbed antiserums were then used in the immunofluorescence studies described in (*7*). Before being applied onto the vessel sections, the antiserums were absorbed with intima-media preparation at 37°C for 2 hours and cleared in a microcentrifuge (Brinkmann).
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Genetic Variation in the Human Insulin Gene

Abstract. *Four recombinant lambda phages containing nucleotide sequences complementary to a cloned human preproinsulin DNA probe have been isolated from human DNA. Restriction analyses in conjunction with Southern hybridizations reveal two types of gene sequences. One isolate of each type was subjected to complete nucleotide sequence determination. The sequences contain the entire preproinsulin messenger RNA region, two intervening sequences, 260 nucleotides upstream from the messenger RNA capping site, and 35 nucleotides beyond the polyadenylate attachment site. Our results strongly suggest that these two gene types are allelic variants of a single insulin gene.*

Insulin is a polypeptide hormone, consisting of two chains (A and B) that are linked by disulfide bonds. Much is known about the translational and posttranslational events that occur in the biosynthesis of this molecule. The A and B chains are synthesized as a single precursor polypeptide, joined together by a connecting peptide (C peptide). This proinsulin molecule is contained within an even larger precursor molecule (preproinsulin), which includes an additional amino terminal extension or signal peptide to direct the insulin precursor to the cell's secretory machinery. Little is known, however, about the transcription of the insulin gene and posttranscriptional processing of the primary transcript in the pancreatic beta cell. Recombinant DNA technology permits the isolation of single copy chromosomal genes and thus facilitates the experimental approach to investigating these problems. We report here the isolation and characterization of two recombinant lambda phages containing human preproinsulin gene sequences.

A human chromosomal DNA library (*1*) was prepared by partially digesting fetal liver DNA with the restriction endonucleases Hae III and Alu I. The resulting DNA fragments were inserted into phage lambda Charon 4A in a manner which permits excision of the inserted fragment from the phage DNA by Eco RI restriction endonuclease cleavage (*2*). We screened 600,000 recombinant phages and identified four independent isolates containing sequences complementary to a ^{32}P-labeled cloned human preproinsulin complementary DNA (cDNA) probe (*3*). The four recombinant lambda phages were characterized by restriction endonuclease cleavage analysis in conjunction with Southern hybridization (*4*). When the restriction enzyme Pst I was used, the four phages displayed two types of hybridization patterns: two isolates (alpha type) contained hybridizing fragments of length 900, 600, and 310 base pairs; and two (beta type) contained hybridizing fragments of length 900, 520, 310, and 80 base pairs (data not shown).

Specific hybridization probes, representing various regions of the cloned cDNA, were used to correlate the position of these Pst I fragments within the physical map of the human insulin gene. A probe containing sequences from the 3′ end of the preproinsulin cDNA clone hybridized to the alpha-600, beta-520, and beta-80 fragments, localizing the alpha- and beta-type Pst I cleavage differences to the 3′ region. The alpha- and beta-900 base-pair fragments were mapped to the middle of the preproinsulin coding region and the alpha- and beta-310 fragments were mapped to the 5′ end region.

In the rat, mouse, and several fish species there are two insulins (I and II), which are the products of nonallelic genes (*5*); these two insulins are almost identical in their amino acid sequences. In the rat, however, where the two insulin genes have been isolated and sequenced (*6*), the structure of the two genes is different. In brief, the rat I gene has one intervening sequence, whereas the rat II gene has two intervening sequences (*6*). To detect fine-structure differences between the alpha and beta sequence types of human insulin genes, we

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