Adhesion of Blood Platelets to Subendothelial Surface: Distinct from Adhesion to Collagen

In the formation of an intravascular thrombus or hemostatic plug, the initial response is platelet adhesion. There is abundant in vitro evidence that platelets adhere to collagen, and that they subsequently aggregate[1]. Accordingly, collagen has been considered to be the vascular substrate in hemostasis[2]. We have evidence[3] that a major component of the material underlying the endothelial cell of large vessels is non-collagenous in nature; rather it resembles the microfibrils characterized by Ross and BORNSTEIN[4]. In the present report, data are given suggesting that platelet adhesion to the subendothelium of larger vessels differs in important respects from that observed with collagen.

Platelets adhere to a vessel wall where it is denuded of endothelium[5], and in capillaries the endothelial basement membrane is the reactive surface[6]. Large arteries have no continuous basement membrane; electron microscopy reveals instead microfibrils, elastic tissue and amorphous material in close association with the plasma membrane of endothelium (Figure 1 A and B). Within minutes after selective removal of the endothelium by a balloon catheter as described below, platelets adhere and spread on these structures to from a tight and continuous 'pseudoendothelium' (Figure 1 C and D)[7].

To investigate the platelet reactivity of the subendothelial surface, an in situ isolated aortic vessel perfusion-system was constructed as follows: The aorta of an anesthetized rabbit was ligated 2 cm distal to the renal arteries. Proximal and distal to this ligature the aorta was cannulated with Silastic tubing (Dow Corning, 1.58 mm ID, 2.45 mm OD). From the proximal tubing 40 ml of blood were collected into different anticoagulants. Then, as the abdominal aorta was perfused at 120 mm Hg with Ringer's solution (pH 7.4) at 37 °C through the distal cannula, the rabbit was exsanguinated from the proximal tubing. After the abdominal aorta was washed for 5–7 min, a balloon catheter was introduced into a femoral artery, inflated with air to 300 mm Hg, moved up to the distal tubing, and withdrawn. This procedure caused selective denudation of endothelium[7]. During continuous perfusion with Ringer's, the aorta was cannulated with a third segment of Silastic just proximal to the aortic bifurcation, and all tributaries of this isolated aortic segment were ligated. Such segments, 3–5 cm long, were perfused with native or heparinized blood by connecting the distal tubing with the aorta, and the bifurcation tubing with the vena cava of another untreated or heparinized rabbit (500 U Heparin/kg body weight; blood coagulation time >1 h). Autologous anticoagulated blood or platelet rich plasma (final concentrations of anticoagulants used: Heparin 10 U/ml; Na-citrate 0.32%, EDTA 0.1%) was perfused by a proportioning pump circulating blood or plasma from a reservoir via a heating coil through the aortic segment and back to the reservoir. All blood and plasma perfusions

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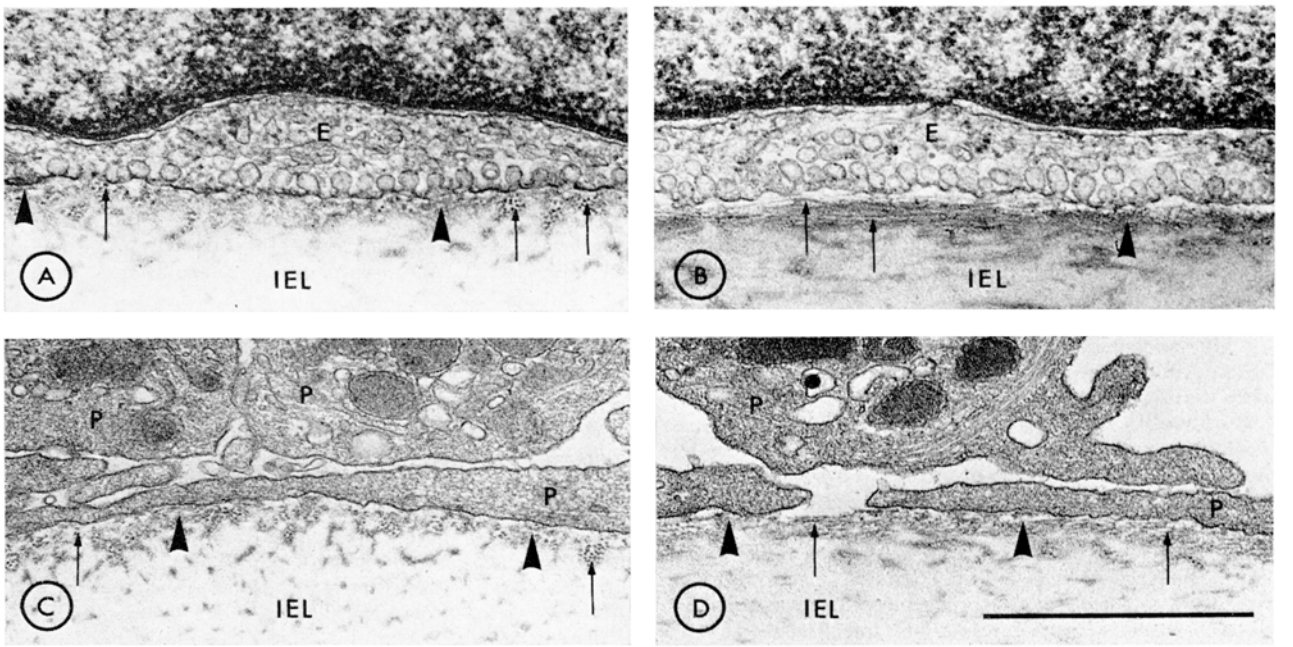

Fig. 1. Electronmicrographs of rabbit aorta. Normal wall is shown in (A) cross and (B) longitudinal section. An endothelial cell (E) with nucleus and pinocytotic vesicles is seen in close contact with the subendothelial surface. 3 morphologic components can be distinguished in the subendothelial area: Electron dense microfibrils (→), approximately 110 Å wide and oriented longitudinally; electron-lucent elastic tissue of internal elastic lamina (IEL); amorphous electron dense material (►) which is irregularly distributed. (C) in cross and (D) in longitudinal section show platelets (P) adhering to the subendothelial surface after denudation of endothelium and exposure to native blood. ×35,000.

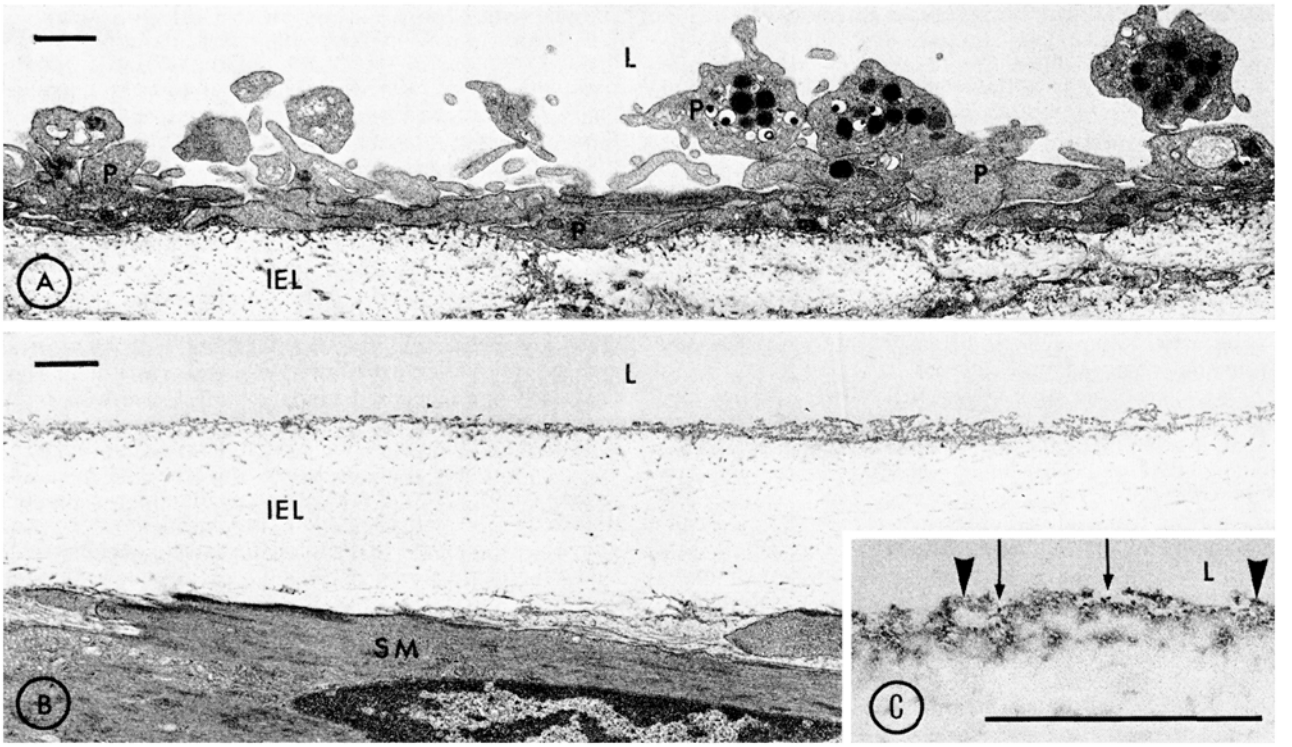

Fig. 2. Electronmicrograph of rabbit aorta denuded of endothelium and subsequently perfused with (A) citrated blood, or (B and C) citrated platelet-rich plasma. A tight platelet layer forming a 'pseudoendothelium' is seen in (A), whereas in (B) the subendothelial surface remains naked. Insert (C) shows the naked subendothelial surface at higher magnification. Additional symbols: L, lumen; SM, smooth muscle cell of media. A and B ×8,000; C ×36,500.

were of 10 min duration and were maintained at 37 °C. Immediately after exposure to blood or plasma, the aortic segment was fixed by perfusion with 2.5% Glutaraldehyde in 0.1 *M* phosphate buffer pH 7.4 at 37 °C. The flow velocity of all perfusates used was similar, and amounted to about 10 ml/min. Platelet counts were taken from the reservoir before, and from the bifurcation tubing after perfusion. A middle portion of the aortic segment was processed for electron microscopy, distal and proximal portions for light microscopy. The extent of platelet adhesion was evaluated in cross sections by light microscopy; electron microscopy was used for qualitative differences, and as confirmation of findings at the light level.

After perfusion of the denuded aorta with native blood, a tight and continuous platelet layer formed within 10 min, and in several specimens mural platelet thrombi with diameters up to 100 μm were encountered. After perfusion with heparinized or citrate whole blood, similar findings were observed, although the thrombi were less prominent in the presence of citrate. These observations demonstrate the reactivity of the subendothelium even after prolonged washing.

Experiments with heparinized specimens were discontinued because of massive aggregation of free platelets, a reaction not seen in the other blood preparations, the platelet count of which remained unchanged. Platelet adhesion was influenced by the low concentration of divalent cation, since EDTA-anticoagulated whole blood gave markedly diminished adhesion, with large areas of the subendothelial surface remaining platelet-free. Whole blood evidently provided a contributory factor or factors, since citrated platelet-rich plasma perfusion was followed by little or no platelet adhesion. The citrate itself was not an effective inhibitor of adhesion, since citrated whole blood gave abundant platelet adhesion. The above observations are illustrated in Figure 2 and documented in the Table.

The adhesion of platelets to the subendothelial surface appears to be influenced by: a) *The surface itself* which consists of at least non-collagenous microfibrils, elastin and amorphous material; most likely the variety of biochemical entities is far greater. Evidently Ca^{++} or Mg^{++} are required for platelet adhesion to some of these materials, since EDTA was inhibitory. Adhesion of platelets in EDTA platelet-rich plasma was readily

Platelet adhesion to subendothelial surface of rabbit aorta

Perfusate	No. of aortas studied	Extent of platelet adhesion P0	P1	P2
Native blood	4	0	0	16
Heparinized blood	3	0	0	12
Citrated blood	3	0	1	11
EDTA blood	3	1	11	0
Heparinized PRP	1	0	4	0
Citrated PRP	4	13	3	0

Two randomly selected cross sections of each distal and proximal portion of perfused vessel segment were examined by light microscopy. P0, platelets absent; P1, few platelets adhering; P2, loose-tight continuous platelet layer.

demonstrable to fragments of rabbit Achilles tendon. b) *Factors in whole blood* which are missing from platelet-rich plasma, as shown in specimens perfused with citrated preparations. Whether this is HELLEM's[8] factor 'R' of red cells which was later identified as ADP[9], or a labile protein inactivated during preparation of platelet-rich plasma, or a rheologic phenomenon remains to be established.

These considerations are in contrast to the platelet-collagen adhesion reaction which occurs equally well in whole blood or platelet-rich plasma with or without divalent cation. Studies on the mechanism of platelet adhesion to collagen is thus not sufficient for complete understanding of platelet hemostatic reactions; other biologic materials, such as non-collagenous microfibrils, and factors influencing platelet adhesion, such as red cells, deserve additional attention.

Zusammenfassung. Das subendotheliale Gewebe von Kaninchenaorten besteht zur Hauptsache aus Mikrofibrillen, Elastin und amorphem Material. Die Adhesion von Blutplättchen an subendothelialem Gewebe ist von der Präsenz von Erythrozyten und chelierbaren Ionen abhängig. Dies steht im Gegensatz zur Adhesion von Plättchen an Kollagen.

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Effect of Stimulation on Synaptic Vesicles in the Superior Cervical Ganglion of the Cat

Physiological[1,2], morphological[3,4] and biochemical[5] evidence suggests that synaptic vesicles may play an important role in the storage and release of neurotransmitter substances. Furthermore, several investigators[6–8] have recently reported that prolonged (15–90 min) stimulation can under certain conditions cause a reduction in the number of vesicles in cholinergic nerve terminals. In some of these latter experiments evidence of transmission failure was obtained and this phenomenon appeared to be related to the depletion of synaptic vesicles. However, no attempt was made to correlate these changes in the vesicle population with the tissue content of acetylcholine (ACh).

Since we[9] had previously demonstrated that preganglionic stimulation at 60/sec for 4 min could reduce the ACh content by about 30%, we thought it would be of interest to ascertain what effects such a short period of stimulation would have on synaptic vesicles in the cat's superior cervical ganglion. If these vesicles are the storage sites for ACh, then one might expect that a significant depletion of ACh would be accompanied by a corresponding reduction in the number of vesicles.

Methods. Cats weighing 1.5–2.5 kg were anesthetized with α-chloralose i.p. (80 mg/kg). Superior cervical ganglia were exposed by careful dissection so that the natural blood supply to these tissues was preserved. Preganglionic nerve trunks were stimulated at a frequency of 60/sec with pulse durations of 2 msec. The voltage (5–10 V) was adjusted to obtain a maximum response as judged by the degree of mydriasis and the isometric contractile response of the nictitating membrane. Ganglia were fixed by perfusion via the carotid artery with 2% (w/v) glutaraldehyde and were post fixed in 1% (w/v) osmium tetroxide.

Results and discussion. Electronmicrographs of control unstimulated nerve endings are presented in Figures A and B. These pictures reveal presynaptic nerve terminals with their normal content of vesicles and Figure B also illustrates the typical structure of mitochondria which are frequently found in these nerve endings[10]. Preganglionic stimulation at 60/sec for 4 min did not cause any apparent failure of ganglionic transmission, but did induce marked alterations in the ultrastructure of nerve endings and a 30% reduction in the ACh content. Invariably this brief period of stimulation caused a depletion of synaptic vesicles and many of those remaining appear to have lost their characteristic conformation (Figures C and D). In addition, these stimulation parameters frequently caused the swelling and disruption of mitochondria in presynaptic nerve endings (compare mitochondria in Figures B and D). Other investigators[7,8] have also reported similar changes in mitochondria, but they stimulated nerves for a longer period of time (15–90 min). In this regard, we found that stimulation for 4 min at 5/sec did not reduce the ACh content nor did this lower frequency of stimulation induce significant alterations in the fine structure of nerve terminals.

In several preliminary experiments ganglia, which had previously been stimulated at 60/sec for 4 min, were allowed to rest for 2 min in order to determine whether the number of vesicles would increase toward control values. Although the ACh content was restored during this rest period, many nerve endings still had not recovered their quota of vesicles. In view of these latter results the data must be interpreted with caution.

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