Correlation of Plasma Serotonin Changes with Platelet Aggregation in an in Vivo Dog Model of Spontaneous Occlusive Coronary Thrombus Formation

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SUMMARY. The role of platelets in contributing to occlusive coronary artery thrombus formation remains unresolved. A large number of studies have utilized in vitro techniques to study platelet aggregation. This report describes a model of spontaneous in vivo thrombus formation which involves application of current in the left circumflex coronary artery of the dog. Changes in mean coronary blood flow velocity (50% above control) are used to predict the point at which current can be discontinued without interrupting the ongoing process of thrombus formation. Thrombus formation proceeds to total vessel occlusion within 62±18 minutes after discontinuation of current. Coronary sinus plasma serotonin concentrations are used as an in vivo index of platelet aggregation during thrombus formation. Plasma serotonin levels increased only slightly above baseline levels during initial thrombus formation. Coronary sinus serotonin levels rose markedly after cessation of current, reaching a peak just prior to total vessel occlusion. The marked increase in serotonin concentration observed in the latter stages of thrombus formation strongly suggests that platelet aggregation is a significant factor in the evolution of an occlusive coronary thrombus.

CORONARY artery thrombosis has a significant impact on morbidity and mortality in patients with coronary artery disease. Recent angiographic (Rentrop et al., 1981; Mathey et al., 1981) and intraoperative (DeWood et al., 1980) studies have demonstrated that a thrombus superimposed on a stenotic lesion usually is responsible for the occlusion of the vessel supplying the affected area during the course of acute myocardial infarction. Further, some cases of sudden cardiac death may result from occlusive thrombosis of a large coronary artery (Schaffer and Cobb, 1975) or from disruption of a proximal coronary thrombus and distal embolization of platelet aggregates (Haerem, 1972).

Considerable attention is being directed toward understanding the role of platelet aggregation in influencing the process of thrombus formation. Progress has been made in elucidating the role of platelet aggregation during this process. Much of this work has been done in vitro, and even the most sophisticated techniques cannot duplicate the complex interactions that occur in vivo. Several in vivo models of coronary artery thrombosis have been reported (Salazar, 1961; Kodenat et al., 1972; Romson et al., 1980). Salazar showed that coronary thrombosis can be induced by the application of anodal current to the intravascular lumen via a stainless steel electrode positioned under fluoroscopic guidance (Salazar, 1961). Romson et al. (1980) modified this approach by placing a silver wire in the lumen of the left circumflex coronary artery during open-chest procedures to induce coronary thrombosis. Although these methods produce an occlusive thrombus, the continuous application of current precludes the study of mechanisms of platelet aggregation involved during spontaneous thrombus formation.

Studies to delineate the mechanisms of platelet aggregation involved during thrombus formation are hampered by the lack of reliable in vivo indices of platelet aggregation. Detection of circulating platelet aggregates was proposed as evidence of platelet activation in vivo, but this test is difficult to standardize and reproduce (Packham, 1978). Recent efforts to detect platelet activation in vivo have focused on radioimmunoassays of platelet secretory products such as platelet factor 4, β-thromboglobulin and the arachidonic acid metabolite, thromboxane B2 (TXB2). Studies using platelet factor 4 (White and Marouf, 1981) or β-thromboglobulin (Rubenstein et al., 1981; Pumphrey and Dawes, 1982) as markers for intravascular platelet activation have produced equivocal results. This probably is due to sampling problems which may release these products from platelets. Similar artefactual changes due to sampling, combined with imprecise assay meth-
ods, have cast doubt on the validity of using TxB2 as a marker for in vivo platelet aggregation (Fitzgerald et al., 1983; Pedersen et al. 1983). To assess the involvement of platelets during in vivo thrombus formation, more sensitive and specific indices of in vivo platelet aggregation combined with improved sampling techniques are needed.

This report describes a modified electrothrombo-genic method which induces spontaneous thrombus formation in the circumflex coronary artery of dogs. The development of coronary thrombus is followed by monitoring the changes in the velocity of coronary blood flow with predictable times for the evolution of total coronary occlusion. Serotonin is released from the intracellular, electron-dense granules during irreversible platelet aggregation (White, 1979). Studies were designed to determine whether changes in plasma serotonin concentration, due to platelet release of serotonin, can be used as an in vivo index of platelet aggregation in this model of thrombus formation.

Methods

Surgical Preparation

Twenty-eight mongrel dogs of either sex, weighing 17–25 kg, were used. Under pentobarbital anesthesia (30 mg per kg, iv) the dogs were intubated and ventilated with room air delivered by a Harvard respirator. Tidal volume and respiratory rates were adjusted to maintain blood gases and pH within physiological limits. A Millar catheter was inserted via the femoral artery and advanced above the diaphragm to monitor blood pressure. A left thoracotomy was made through the 5th intercostal space, and the heart was suspended in a pericardial cradle. A heparin-bonded Silastic catheter (0.02 inch i.d.) was placed antegrade to blood flow in the coronary sinus 12–15 mm proximal to the point of entry of the coronary sinus into the right atrium. The catheter position was checked by the blood PO2/PCO2 concentrations. A PO2 value of <30% was considered acceptable in assuring that the catheter tip was not sampling mixed venous blood from right atrium. PO2 values of 21–27% were usually obtained.

The circumflex artery was exposed by blunt dissection at the distal border of the left atrial appendage, and the vessel size was determined by direct measurement. A Doppler flow probe connected to a 20-MHz pulsed Doppler module (Bioinstrumentation Laboratories, Baylor College of Medicine) (Hartley et al., 1978) was placed around the circumflex artery. Proximal to the flow probe (within 5–7 mm), a 25-gauge silver wire was inserted into the arterial lumen and anchored. The wire was modified by the addition of “wings” just distal to the right angle turn of the exposed tip that is inserted into the lumen of the vessel (Fig. 1A). These “wings” permit anchoring of the wire to the epicardium and also maintain its placement. This wire serves as the anode to stimulate thrombus formation. A ground wire to complete the circuit was implanted subcutaneously on the chest wall. A heparin-bonded Silastic catheter (0.04 inch i.d.) was placed in the left atrial appendage. An inflatable vascular occluder was placed proximal to the thrombus stimulation needle.

In some experiments, a second Doppler flow probe was placed proximal to the thrombus stimulator needle to measure the changes in the velocity of blood flow proximal to the evolving thrombus (Fig. 1A). In another set of experiments, a PE-60 catheter connected to a Statham P-23D6 pressure transducer was placed in one of the distal branches of the circumflex artery to measure pressure in the arterial tree during thrombus development.

Measurement of the Velocity of Coronary Blood Flow

The distal Doppler flow probe must be placed within 5–7 mm of the thrombus stimulator needle entry point to measure the change in blood flow velocity (Fig. 1A). There was a close relationship between frequency shift (in kHz) obtained from the Doppler flowmeter and actual blood flow, as measured by collecting timed samples from a catheter advanced retrograde to a point just distal to the flow probe in separate calibration experiments. However, we found that the percentage change in blood flow velocity as measured by frequency (kHz) was an accurate predictor of the point (50% increase over control) where current could be stopped and spontaneous occlusion would proceed. Therefore, the blood flow velocity data are reported in kHz. Since pulsed Doppler is range-gated, the velocity of blood flow in kHz/mm is detected at a selected distance from the crystal face (Hartley et al., 1978) which optimizes near the center of the vessel. During thrombus development, the blood flow is axially displaced (Fig. 1B). Therefore, the range gate is continually adjusted to record the maximum velocity of blood flow as thrombus formation progresses. Unlike the electromagnetic flow probe, the Doppler flow probe operating at 20 MHz of high frequency sound is unaffected by the low current applied to the stimulator needle.
Experimental Protocol

After instrumentation, dogs were allowed to stabilize for 30 minutes. Control hemodynamic measurements, including heart rate, blood pressure, and electrocardiogram (ECG), as well as phasic and mean coronary blood flow velocity, were recorded continuously on a Grass recorder. After control readings, the hyperemic response to a 15-second total occlusion was recorded and was repeated every 30 minutes during the course of the experiment. An anodal current of 150 μA was then applied to the thrombus stimulator wire from a 9-V battery connected in series to a variable resistor and ammeter. Blood samples for serotonin measurement were drawn from the left atrium and coronary sinus every 20 minutes or less. Current was stopped when the mean blood flow velocity increased by 50% over control values. Thrombus formation then proceeded spontaneously, with eventual total vessel occlusion.

After total occlusion, monitoring was done for 60–90 minutes to confirm that occlusion of the artery was not a transient phenomenon (i.e., vasospasm). Total occlusion was accompanied by typical ECG changes including T-wave changes, atrial and ventricular arrhythmias, and hypotension. After the 60-minute stabilization period, the coronary sinus catheters were flushed and filled with 0.9% saline. After 30 minutes to confirm that occlusion of the artery was not a transient phenomenon (i.e., vasospasm), the anodal current was applied for 4 hours to the thrombus, and coronary sinus catheters were flushed and filled with saline. After 4 hours, the coronary arteries were allowed to stabilize for 60–90 minutes before another control period was established. Total occlusion was accompanied by typical ECG changes including S-T segment elevations. At the end of the experiment, the heart was rapidly excised and the section of the coronary artery containing the thrombus was dissected free and fixed for 2 hours at room temperature in 2.5% glutaraldehyde (vol/vol)-2% paraformaldehyde (wt/vol) buffered with 0.1 M cacodylate, pH 7.4. Then specimens were washed in 10% sucrose (wt/vol) buffered with 0.1 M cacodylate, pH 7.4, and stored at 4°C in the same buffer. Postfixation was done in 1% osmium tetroxide (wt/vol) in 0.1 M cacodylate buffer at pH 7.4. Dehydration through a graded series of acetone solutions, dehydration, critical point drying, and then sputter-coating with gold and paladium. The samples were examined in a Jeol 100C TEM/SEM electron microscope. In five animals, the section of the coronary artery containing the thrombus and the formed thrombus were dissected out, rapidly frozen to −75°C, and serotonin concentrations were measured in the thrombus as well as in the coronary artery.

The same protocol was followed for partial thrombus formation experiments, except that the current was stopped when the mean blood flow velocity was 30–40% above control values. Subsequently, these animals were monitored for 4 hours, the coronary arteries were opened, and the degree of thrombotic occlusion was determined. For sham-operated controls, the same protocol was used, but no current was applied. After 4 hours, the coronary arteries were examined.

Measurement of In Vivo Release of Serotonin due to Platelet Aggregation

Baseline blood samples were collected from the left atrial and coronary sinus catheters following the 30-minute stabilization period. Subsequent samples were collected in pairs every 20 minutes or less throughout the experiment. After onset of total occlusion, samples were drawn every 30 minutes until the end of the experiment. Catheters were flushed and filled with 0.9% saline. After discarding the void volume, a 2-ml blood sample was collected into a plastic tube containing a solution (45 μl/ml of blood) of 4% ethylene diamine tetraacetic acid (wt/vol) and 0.15% imipramine (wt/vol). Prostacyclin (2 μg/ml of blood) was then added. Prostacyclin is unstable in acid pH and stable for about 30 minutes at pH 8.0 (Cho and Allen, 1978). Therefore, prostacyclin is made up in 70% acetonitrile solution and diluted when necessary in phosphate buffer, pH 8.0, containing 5% albumin. Albumin improves the half-life of prostacyclin by 50%. The blood was gently mixed and spun down at 120 g for 20 minutes to obtain platelet-rich plasma. An aliquot of this was transferred to 1-ml conical plastic tubes with a sucrose-albumin (9:1) (vol/vol) "cushion" at the bottom. The tubes were centrifuged at 4000 g for 20 minutes to precipitate the platelets on the "cushion" and to obtain platelet poor plasma for serotonin assay.

The serotonin concentrations in coronary sinus, platelet-poor plasma were determined as an index of ongoing platelet aggregation during thrombus formation. Platelet-poor plasma prepared by standard methods (Hussain and Sole, 1981; Frattini et al., 1979) yielded high and variable values for serotonin levels (Table 1) similar to previously published results. Using the improved method described above for collection and separation of platelet poor plasma, we obtained serotonin levels that were 3- to 4-fold less than the values previously reported (Table 1) (Demet et al., 1978; Frattini et al., 1979; Hussain and Sole, 1981). This improved method was used for these studies.

Serotonin concentrations were measured by modifying the method of Hussain and Sole (1982). The modifications were as follows: The crude hydroxyindole-O-methytransferase was prepared from pineal glands as described, and then further purified by Sephadex gel (G-200) chromatography to obtain the pure enzyme which was stored lyophilized. When reconstituting the enzyme, we used 0.1% albumin containing 1 unit of aprotinin per ml to ensure the stability of the purified enzyme. During the acetylation stage, the excess acetic anhydride present was evaporated (<200 milliTorr) for 10 minutes. This removed the excess acetic anhydride without degrading any of the formed N-acetylsertotonin. These modifications improved the sensitivity of the assay to 2 pg of serotonin and the interassay coefficient of variation to 3.75%.

In Vitro Platelet Aggregation and Correlation with Serotonin Concentrations in Platelet-Poor Plasma

From six dogs, 10 ml of venous blood were collected into plastic tubes with 3.8% citrate (1 ml/10 ml), and platelet-rich plasma was separated by centrifugation at 4°C in a Savant vacuum centrifuge under high vacuum (<200 milliTorr) for 10 minutes. This removed the excess acetic anhydride without degrading any of the formed N-acetylsertotonin. These modifications improved the sensitivity of the assay to 2 pg of serotonin and the interassay coefficient of variation to 3.75%.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Variability of Serotonin Concentration in Platelet-Poor Plasma due to Differences in the Method of Collection*</th>
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<tbody>
<tr>
<td>Blood collected with</td>
<td>Serotonin in platelet-poor plasma collected without albumin-sucrose &quot;cushion&quot; (ng/ml)</td>
</tr>
<tr>
<td>EDTA</td>
<td>4.7 ± 0.6f</td>
</tr>
<tr>
<td>EDTA + imipramine</td>
<td>5.8 ± 1.3f</td>
</tr>
<tr>
<td>EDTA + prostacyclin</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>EDTA + imipramine + prostacyclin</td>
<td>3.2 ± 0.6</td>
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</table>

Results are expressed as mean ± 1 SD and are the means of 10 estimations.

* Coronary sinus blood for preparation of platelet-poor plasma was obtained from dogs with implanted coronary sinus catheters which were placed at least 14–16 days prior to the time of sampling.
† Significantly different at P < 0.01 from the other values within the same group (unpaired t-test).
102 g for 15 minutes at room temperature. After the upper 1/3 of platelet-rich plasma had been removed, the remaining was recentrifuged at 4000 g for 10 minutes to yield platelet-poor plasma. Platelet count in platelet-rich plasma was adjusted to 2 × 10^6/mm^3 with platelet-poor plasma. Platelet aggregation was determined in 0.4 ml of platelet-rich plasma by the method of Born (1962). Aggregation responses to added adenosine diphosphate (ADP) (0.5-10 μM) was quantified as change in light transmission and expressed as percent of maximum aggregation. Another aliquot of platelet-rich plasma (0.4 ml) was used to determine the change in serotonin concentration in platelet-poor plasma during ADP-induced aggregation. The same concentrations of ADP were added, incubated with stirring at 37°C for 5 minutes, and then centrifuged on albumin-sucrose cushion to obtain platelet-poor plasma for serotonin assay. Two types of controls were used: (1) platelet-rich plasma was centrifuged immediately and platelet-poor plasma was collected for serotonin assay, and (2) to platelet-rich plasma, 2 μM, 5 μM, and 10 μM ADP was added and the sample immediately centrifuged, as before, to obtain platelet-poor plasma for serotonin assay.

**Statistical Analysis**

All values are expressed as mean ±1 so. Analysis of variance (ANOVA) was used to determine whether there was a significant difference in the serotonin concentration between the thrombus formation group, partial thrombus formation group, and the control group. Duncan's multiple range test (Zar, 1974) was used to identify the group mean values that differed significantly. Unpaired Student's t-test was used for all the other comparisons. In all cases, a P value <0.05 was considered significant.

**Results**

**Thrombus Formation Studies**

Twenty-eight dogs were instrumented for thrombus formation studies. The circumflex artery diameter varied from 2.5–3.5 mm. The resting hemodynamic variables were similar between all animals. The resting blood flow was 4.6±1.3 kHz. Due to differences in coronary artery size, the changes in the velocity of phasic and mean coronary blood flow

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**Figure 2.** Representative recording of the mean and phasic coronary blood flow velocities (kHz) from a dog during thrombus formation. Note the following: (1) at the time of stopping of the current (50 minutes), the mean blood flow velocity has increased by 50% from its resting value, (2) the change in waveform as thrombus formation progresses, (3) the intermittent, irregular decrease in flow velocity followed by an increase, and (4) 67 minutes after stopping the current, blood flow has ceased (total occlusion). The figure panels read from left to right, top to bottom.
are expressed as a percentage change of the resting values. The maximum baseline hyperemic response to a 15-second total occlusion of the circumflex artery was 340 ± 51% of control blood flow. With thrombus formation, two changes were noted in the distal Doppler flow velocity measurements. First, as current is applied, a unidirectional change in the range gate occurs which is the earliest indication of the developing thrombus. Second, there is an increase in the mean and phasic blood flow velocity (Fig. 2). In experiments utilizing both proximal and distal flow probes, increases in blood flow velocity were seen at the distal probe, while no changes were detected by the proximal probe (Fig. 3). The average time of current application necessary to produce a 50% increase in the velocity of mean blood flow was 59±20 minutes. Current was stopped when the increase in mean blood flow velocity reached a value of 50% above control.

As thrombus formation spontaneously progressed to occlude the vessel, further increases in the velocity of phasic and mean blood flow were noted (Fig. 2). Mean blood flow velocity reached a peak of 254±52% above control value 26±18 minutes after the current was discontinued. After reaching the peak, the increased blood flow velocity recorded distal to the forming thrombus began to decrease (Fig. 3). Concomitantly, a corresponding decrease in blood flow velocity proximal to the thrombus was observed (Fig. 3). Total occlusion of the circumflex artery by the thrombus occurred 62±18 minutes after stopping the current. In six of the 28 animals, thrombus formation was incomplete (subtotal occlusion) during this time period (62±18 minutes). At postmortem, these failures were found to be due to improper placement of the stimulator needle; i.e., in all instances, the needle was not placed parallel to the lumen and in direct contact with the vascular endothelium.

Reactive hyperemic responses to a 15-second occlusion were repeated soon after stopping the current. The reactive hyperemic response was 30±8% below control responses. Subsequent occlusions were not done to avoid dislodging the evolving thrombus in the lumen. Comparative pressure measurements were made in the circumflex artery of four animals during thrombus formation. A catheter, placed in one of the distal circumflex branches, measured the pressure drop across the thrombus as it evolved. The control aortic pressure (systolic/diastolic) was 106±8/86±6 mm Hg and showed an insignificant decrease to 98±5/78±7 mm Hg when total coronary occlusion developed. The control distal artery pressure was 101±6/80±4 mm Hg. At the time of 50% increase in mean blood flow velocity, the distal pressure was 92±7/71±4 mm Hg and not significantly different from the control value. Significant reduction in distal artery pressure occurred only when mean blood flow velocity began to decrease. The distal pressure decreased to 28±3/24±4 mm Hg when total occlusion developed. There was no further decrease in distal pressure when the vascular occluder was inflated to totally occlude the vessel.

**Measurement of Serotonin Release during Thrombus Formation**

Baseline serotonin levels were 8.6±2.7 ng/ml for the left atrium and 10.4±3.6 ng/ml in coronary sinus (Fig. 4). The difference between the two values was

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Representative experiment of the time course of changes in coronary blood flow in both the proximal (Δ) and distal (○) Doppler flow probes and coronary sinus plasma serotonin concentrations (●) during thrombus formation and total occlusion. Control blood flow velocities are represented at 100% (right margin). Current application was discontinued (D/C current) when mean blood flow increased 50% above control in the distal flow probe (○).
not statistically significant. These levels are higher than the values obtained from dogs with chronically implanted catheters who had been allowed 2 weeks recovery from surgery (Table 1). This is due to platelet activation that occurs during surgery. During application of current, serotonin concentration in coronary sinus increased from 10.4±3.5 ng/ml to 19.2±8.4 ng/ml (Fig. 4). After the current was stopped, there was a gradual increase in coronary sinus serotonin levels (Fig. 4) which peaked after mean blood flow began to decline (Fig. 3). The maximum coronary sinus serotonin concentration measured was 213.0±63.0 ng/ml (Fig. 4). Coronary sinus serotonin levels rapidly declined after total occlusion of the circumflex artery (Fig. 4). During current application, left atrial serotonin concentration changed from a baseline value of 8.6±2.7 ng/ml to 9.4±2.9 ng/ml. After the current was stopped, the left atrial serotonin concentrations increased further, reaching a peak of 10.9±3.7 ng/ml just before total occlusion of the circumflex artery. After occlusion of the vessel, the left atrial serotonin concentrations decreased. Thus, throughout the study, the atrial serotonin concentrations did not change significantly from baseline values (Fig. 4). Serotonin concentration in the formed occlusive thrombus was 17.2±4.7 µg/g of thrombus (wet weight). Serotonin concentration in the segment of the circumflex artery containing the occlusive thrombus was 804.5±205.8 ng/g of tissue. Serotonin concentration in the partially occlusive thrombus was 2.1±0.7 µg/g of thrombus (wet weight) (n = 5).

Two sets of control experiments were done. One, to determine the effect of surgery and instrumentation on plasma serotonin levels and the other to measure changes in plasma serotonin concentration during partial thrombus formation. In six dogs following instrumentation, no current was applied and serotonin concentrations were measured in left atrium and coronary sinus for 4 hours. No significant changes in plasma serotonin were found (Fig. 4). In another eight dogs, after instrumentation, current was applied until the mean blood flow velocity increased by only 30–40%. Current then was stopped. These animals failed to develop occlusive thrombi, and there was only a small increase in coronary sinus serotonin levels (Fig. 4).

Anagrelide, a potent platelet anti-aggregatory drug (Fleming and Buyniski, 1983), was used to demonstrate the in vivo correlation between platelet aggregation and serotonin release during thrombus formation. Anagrelide was administered (100 µg/kg) to five dogs 30 minutes prior to initiation of thrombus formation. Application of current to the intra-arterial lumen resulted in a 50% increase in blood flow velocity, at which point the current was stopped. During the next 4–5 hours, occlusive thrombus formation did not occur, and the plasma serotonin concentrations did not change from control values. Scanning electron microscopic exami-

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**Table 2**

<table>
<thead>
<tr>
<th>Platelet-rich plasma treated with</th>
<th>Serotonin levels (ng/ml)</th>
<th>% Light transmittance</th>
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<tbody>
<tr>
<td>Control I*</td>
<td>1.04 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Control II† (treated with 10 µM ADP)</td>
<td>1.98 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>0.5 µM ADP</td>
<td>2.21 ± 0.34</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>2.0 µM ADP</td>
<td>6.76 ± 1.02†</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>5.0 µM ADP</td>
<td>37.86 ± 3.14§</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>10.0 µM ADP</td>
<td>48.14 ± 4.64§</td>
<td>76 ± 6</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± 1 SD and are the means of six estimations.

* Platelet-rich plasma was immediately centrifuged and platelet poor plasma was used for assay.
† To platelet-rich plasma 10 µM ADP was added; the sample was immediately centrifuged and the platelet-poor plasma was used for assay.
§ Significantly different at P < 0.005 from controls.
| Time of stopping the current | Onset of total occlusion | Totally occlusive thrombus formation dogs | Partial thrombus formation dogs | Sham thrombus formation dogs |

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**Figure 4.** Changes in serotonin concentrations (mean ± 1 SD during thrombus formation. Maximum increases in serotonin concentration with totally occlusive thrombus (○) are significantly different when compared to partial thrombus (Δ) formation (P < 0.001; ANOVA, Duncan's multiple range test). No significant increases in serotonin concentrations are observed after the time of stopping the current in all groups (arrow), and a rapid decline to near control values (●) is seen in dogs after total occlusion (○). In all dogs, the left atrial serotonin concentrations did not exhibit significant changes throughout the study, and the values were the same as control (sham thrombus dogs) (Δ).
nation of the thrombi from anagrelide-treated dogs revealed a partially occlusive thrombus, with little or no evidence of platelet aggregation when compared to controls.

Correlation of Platelet Aggregation with an Increase in Serotonin Concentration in Platelet-Poor Plasma

The relationship between platelet aggregation and serotonin release was also determined by in vitro aggregometry. Platelet aggregation was induced in platelet-rich plasma by ADP, and the percentage aggregation of platelets correlated with the increase in serotonin concentration in platelet-poor plasma (Table 2). When compared to controls, increasing amounts of added ADP produced higher maximum amounts of platelet aggregation and higher serotonin levels in platelet-poor plasma (Table 2).

Scanning Electron Microscopy

Figure 5A is a representative scanning electron micrograph of the surface of an occlusive thrombus revealing some red cells, fibrin deposition, and numerous clumps of aggregated platelets. In four other dogs, current was applied to the circumflex artery lumen until mean blood flow velocity increased by 50%. These animals were immediately killed and the arterial lumen examined. There was a small, smooth-surfaced thrombus in the lumen, causing a partial occlusion (<50%) of the vessel. Scanning electron microscopy of this thrombus revealed (Fig. 5B) a smooth, essentially platelet-free surface consisting mainly of fibrin.

Discussion

The model of coronary thrombosis used in this study is a modification of that originally described...
by Salazar (1961) and employed by others (Romson et al., 1980). In all these models, thrombus is formed by continuous application of current to the arterial lumen. The principal difference between previous models and ours is that current is discontinued after initiating thrombus formation. This permits study of platelet aggregation during spontaneous thrombus formation and eliminates the influence of continuous electrical stimulation. The monitoring of changes in velocity of blood flow distal to the thrombus is used to predict the time for stopping current (Figs. 2 and 3). The volume of blood flow (Q) in the coronary artery is given by the equation \( Q = V_{avg} \times A \) where \( A \) is the area of the lumen and \( V_{avg} \) is the average velocity of blood flow (Ishida et al., 1983). As the luminal area decreases, to maintain the same volume of blood flow, the velocity must increase. This is seen distal to the thrombus and not proximal to it. We found that the distal flow probe must be within 5–7 mm of the evolving thrombus to detect the increased blood flow velocity. Double-Doppler flow probe studies revealed an acceleration in distal flow velocity while the proximal flow velocity remained unchanged (Fig. 3). This suggests that the acceleration in blood flow seen distal to the thrombus is caused by the thrombus partially occluding the vessel and accelerating the stream of blood flow (Fig. 1B). The flow velocity across a stenosis will also be influenced by the pressure differential across the stenosis. In some experiments, pressure changes were measured across the stenosis as it evolved. As velocity of blood flow increased distal to the thrombus, a pressure drop did not develop across the thrombus. The distal arterial pressure declined only when the increased blood flow velocity distal to the thrombus began to decline. In addition, the baseline hyperemic response was compared with one obtained just after stopping the current. There was a 30 ± 8% decrease compared to the control value. Based on previous studies by Gould (1978) and Klocke (1983), this indicates that the percentage occlusion of the circumflex artery at the time of discontinuing the current must be around 40–50%. This was confirmed by direct observation; i.e., some animals were killed immediately after the current was discontinued and artery inspected. An approximate 40–45% occlusive thrombus was present in the lumen of the vessel.

In partial thrombus formation studies (Fig. 4) where mean blood flow was increased only 30–40% over control, the thrombus did not extend spontaneously and, at postmortem, only a 25–30% occlusive thrombus was present. These differences suggest that a certain percentage occlusion of the circumflex artery must be present before spontaneous "growth" of the originally formed thrombus can take place due to platelet aggregation. This would be similar to the concept in coronary artery disease where a critical narrowing of the vessel by an atherosclerotic lesion or focal vasoconstriction sets the stage for platelet aggregation and release of vasoconstrictor substances. This in turn promotes further platelet aggregation and progressive coronary narrowing, leading eventually to occlusive thrombus formation (Willerson et al., 1984).

During irreversible platelet aggregation, serotonin is released from the dense granules, which results in increased plasma serotonin levels. However, the serotonin concentration in plasma cannot be used as an index of platelet aggregation because intact platelets will rapidly reaccumulate the serotonin present in plasma (Baumgartner and Born, 1978). Imipramine inhibits serotonin uptake by platelets (Grant and Zucker, 1979). When platelet aggregation was induced in platelet-rich plasma in the presence of imipramine, there was an increase in serotonin concentration in platelet-poor plasma (Table 2). However, with imipramine, the accepted methods for preparation of platelet-poor plasma produced a high degree of variation in plasma serotonin levels (Table 1). This is probably due to imipramine inhibition of platelet serotonin uptake and variable platelet aggregation induced during preparation of platelet-poor plasma. For these reasons, prostacyclin was used to inhibit platelet aggregation during the collection process. These modifications resulted in a 3- to 4-fold lowering of serotonin values in platelet-poor plasma with minimal variation in serotonin values with repeated sampling (Table 1).

In all the studies of thrombus formation, left atrial serotonin levels did not increase significantly, irrespective of the changes in coronary sinus serotonin concentrations (Fig. 4). In fact, when coronary sinus samples exhibited high concentrations of serotonin, right atrial samples had minimal increases, suggesting the rapid reuptake of serotonin from plasma. During current application, only minimal increases in serotonin values were seen (Fig. 4). As thrombus formation continued to total occlusion (post-current), high concentrations of serotonin were observed in the coronary sinus (Fig. 4). This suggests a significant role for platelet aggregation during the latter, occlusive stages of an evolving thrombus. The high concentration of serotonin in the occlusive thrombus and segment of circumflex artery containing the thrombus demonstrates a significant amount of platelet deposition at the thrombus formation site. This, in turn, is reflected by increased coronary sinus serotonin levels. The rapid decline in serotonin levels following total occlusion (Fig. 4) further supports this relationship, since it is unlikely that further platelet aggregation and "wash-out" would occur from the thrombus formation site.

The possibility that the changes in serotonin concentrations were due to decreases in coronary sinus blood flow and/or local blood turbulence and platelet aggregation as a result of mechanical obstruction at the thrombus site were explored. In four separate experiments, instead of current application, the vascular occluder was gradually inflated over a 120-
minute period to occlude the artery. Serotonin concentrations in coronary sinus remained at control levels. When the vessel was opened, there was no evidence of thrombus formation at the site of the vessel stenosis. This excludes the possibility that mechanical occlusion of the vessel alone is likely to cause platelet activation and serotonin release. This also obviates the possibility that the significant increases in coronary sinus serotonin levels during thrombus formation are due to a decrease in total coronary sinus blood flow per se.

Scanning electron microscopy of the occlusive thrombi revealed numerous platelet aggregates covering the surface of the thrombus (Fig. 5A). This is in marked contrast to the partial thrombus formed during application of current which shows a smooth surface, consisting mainly of fibrin and few red cells (Fig. 5B). The specific mechanisms underlying thrombus formation and extension are incompletely understood. In this model, there are two potential mechanisms for spontaneous thrombus extension. Platelet aggregation can be promoted by abnormalities of the vascular endothelial surface (Mustard et al., 1981). The initial fibrinous, thrombotic mass produced by application of current presents such an abnormal surface. Although the insertion of the needle and application of current produces endothelial denudation and damage to the vessel wall (Romson et al., 1980), this alone did not lead to spontaneous growth of the formed thrombus. A critical luminal narrowing appears to be necessary before thrombus formation can progress without external current application. Equally important is the observation that external, mechanical occlusion of the vessel alone did not promote thrombus formation. Thus an interaction between luminal narrowing and abnormal vascular endothelial surface appears to be necessary for a thrombus to propagate itself spontaneously and eventually occlude the vessel. Agrelide prevented the formation of occlusive thrombus in treated dogs with no increases in plasma serotonin levels above control values. However, agrelide did not prevent the formation of the nonocclusive thrombus during current application. All of the above data suggest a close relationship between platelet aggregation, spontaneous thrombus formation, and the release of large amounts of serotonin into the coronary circulation during in vivo platelet aggregation. Further, the serotonin concentration rapidly decreases by the time the coronary blood reaches the systemic circulation, by dilution and/or reuptake by intact, circulating platelets.

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References

Bevers SJ, Knolwes RG, Pogson CI (1983) A sensitive radiometric assay for tryptophan hydroxylase applicable to crude extracts.
Pumphrey CW, Dawes J (1982) Plasma beta-thromboglobulin as

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a measure of platelet activity. Effect of risk factors and findings in ischemic heart disease and after acute myocardial infarction. Am J Cardiol 50: 1258–1261


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