Effect of Aspirin and Sodium Salicylate on Thrombosis, Fibrinolysis, Prothrombin Time, and Platelet Survival in Rabbits With Indwelling Aortic Catheters

By M. Cattaneo, A. Chahil, D. Somers, R. L. Kinlough-Rathbone, M. A. Packham, and J. F. Mustard

We have studied the effect of different doses of aspirin on platelet function, PGI₂ formation, platelet survival, thrombosis, fibrinolysis, and prothrombin time in rabbits with indwelling aortic catheters. The thrombi formed around indwelling aortic catheters were found to have a large fibrin component, and their formation was inhibited by heparin administration. Thus, in these experiments we examined the effect of aspirin (a weak inhibitor of thrombin-mediated platelet aggregation) under conditions in which thrombin was a major factor in the initiation and growth of the thrombi. Only very high doses of aspirin tended to inhibit thrombus formation over the 5-day period of observation, and a statistically significant inhibition of thrombus formation was produced by equivalent concentrations of sodium salicylate. The failure of high doses of aspirin to achieve a significant inhibition of thrombosis is associated with prolongation of the one-stage prothrombin time and increased whole blood fibrinolytic activity; doses of aspirin that inhibited platelet aggregation in response to sodium arachidonate or collagen, and PGI₂ formation by the vessel wall, did not have a significant effect on the amount of thrombus present at 5 days. However, the high doses of aspirin that inhibited PGI₂ formation were associated with a tendency to increased thrombus formation during the first 3 hr after insertion of the catheter. The results of these experiments show that when thrombin is an important factor in the formation of thrombi, aspirin is a weak inhibitor of thrombosis unless doses are used that provide sufficient salicylate to interfere with blood coagulation and promote whole blood fibrinolytic activity. These results also show that thrombus formation can be inhibited without an apparent change in platelet survival.

SOME INVESTIGATORS have reported inhibition of thrombosis in experimental animals receiving aspirin, whereas in other experiments, aspirin has been found to have little inhibitory effect on thrombosis when a vessel wall is severely injured. Since several pathways may be involved in the initiation and growth of thrombi, aspirin may only be inhibitory if the generation of thromboxane A₂ plays a major part in thrombus formation. In circumstances in which thrombin makes a significant contribution to thrombosis, aspirin would not be expected to be inhibitory because this drug has little effect on thrombin-induced platelet aggregation and release and does not influence fibrin formation unless sufficiently high doses are used to inhibit synthesis of vitamin K-dependent clotting factors. In addition, doses of aspirin that inhibit PGI₂ formation may promote thrombosis under some conditions.

Shortened platelet survival is thought to be associated with vessel wall injury and thrombosis. The observation that aspirin inhibits some forms of thrombosis but is without effect on platelet survival indicates either that there may not be a direct relationship between thrombosis and platelet survival or that the type of thrombi that are associated with shortened platelet survival are not influenced by aspirin.

We have found that neither removal of the endothelium from a rabbit aorta with a balloon catheter (which results in a monolayer of platelets on the subendothelium) nor a single balloon-catheter-induced injury to the neointima (which is associated with the formation of platelet-fibrin thrombi) shorten platelet survival. However, continuous injury of rabbit aorta with an indwelling aortic catheter causes the formation of large platelet-fibrin thrombi, and platelet survival is reduced. Thus, although a single injury cannot be used to study the relation among vessel injury, thrombosis, platelet survival, and the effects of aspirin, continuous injury provides an experimental approach for such a study. We have caused continuous injury with an indwelling catheter in the rabbit aorta to examine whether there are doses of aspirin that lessen the size of thrombi that have a large fibrin component, and the effect of such doses of aspirin on platelet survival.

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MATERIALS AND METHODS

Animals
Male New Zealand white rabbits weighing between 2.4 and 3.3 kg were used. Animals of approximately the same weight were used within an experiment.

Drugs
Aspirin (acetylsalicylic acid, Sigma Chemical Co., St. Louis, Mo., no. A-5376) was suspended in distilled water and dissolved by slow addition of a solution of Na2CO3. The pH of the solution was maintained below 6.8 to minimize spontaneous hydrolysis. Aspirin solutions (100 mg/ml or 10 mg/ml) were stored at 70°C and used within 15 days. Sodium salicylate (Fisher Scientific Co., Fairlawn, N.J., no. S-395) was dissolved in distilled water and stored at 20°C. Heparin (Hepalan) was purchased from Harris Laboratories, Brantford, Ontario.

Preparation of Suspensions of Washed Platelets
Suspensions of washed platelets from rabbits were prepared as previously described from blood collected into acid-citrate-dextrose (ACD) anticoagulant. The platelets were labeled in the first washing solution for 60 min at room temperature with Na251CrO4 (Amersham/Searle, Arlington Heights, Ill., 200-500 μCi/μg of chromium; 150 μCi of 51Cr were used to label platelets obtained from the blood of each rabbit). The platelets were then washed once in calcium-free Tyrode solution containing 0.35% bovine serum albumin (Pentex, Fraction V, Miles Laboratories, Elkhart, Ind.) and resuspended in platelet-poor plasma.

In the studies of PGI2-like activity, platelets were prelabeled with 14C-serotonin as 5-hydroxytryptamine-3-14C-creatinine sulfate (Amersham/Searle, 50 μCi/μmole serotonin); 1-2 μCi was used to label platelets obtained from one rabbit.

Insertion of Indwelling Aortic Catheters
The right femoral artery of rabbits anesthetized with sodium pentobarbital (30-40 mg/kg) was isolated. A polyethylene catheter (PE 90, Clay Adams; Becton, Dickinson and Company, Parsippany, N.J.) sealed at the tip with wax was introduced via the femoral artery so that it extended 12-14 cm into the aorta and reached the aortic arch. The femoral artery was ligated with the catheter in place and the incision closed with silk sutures. In sham-operated animals, the aortic arch. The femoral artery was ligated with the catheter in place and the perfusion maintained at the same pressure for 10-15 min. The right femoral artery of rabbits anesthetized with sodium pentobarbital and given an injection of heparin (300 U/kg) before

Measurement of Thrombus Weight
The animals were preanesthetized with Ketamine (Ketaset, Rogar/STB, Division of BTA Products, London, Ontario; 100 mg intramuscularly) and atropine (0.1 mg subcutaneously) 20 min before they were anesthetized with sodium pentobarbital (20-30 mg/kg, intravenously). The left carotid artery and the left femoral artery were cannulated with polyethylene tubing (PE 190). The rabbits were given an injection of heparin (1000 U, intravenously) and were then perfused, via the carotid cannula, with Locke's-Ringer solution (37°C) containing heparin (1 U/ml) at a pressure of 70-100 mm Hg. When the solution draining from the femoral artery was almost clear of red blood cells, the perfusion fluid was changed to 4% paraformaldehyde at 37°C. When paraformaldehyde was detected draining from the femoral artery, the vessel was clamped and the perfusion maintained at the same pressure for 10–15 min. The carotid artery was clamped and the carcass stored at 4°C for 12-18 hr. The aortae were dissected free of extraneous tissue, removed, and cut into 2.5-cm lengths. Each segment of aorta was then slit longitudinally and the thrombi associated with the catheter and injured vessel wall above the aortic bifurcation (excluding thrombus in the femoral artery) were isolated, placed in preweighed plastic dishes, and dried overnight in an oven at 37°C and weighed.

Platelet Survival
The 51Cr-labeled platelets (1.0–1.5 x 108 in a volume of 5 ml) were injected 2 hr before the insertion of the indwelling catheters, and samples of blood (1.5 ml) were collected from an ear vein into 0.5 ml ACD 2, 20, 26, 44, 68, and 92 hr after the injection of the 51Cr-platelets. The 2-hr sample was taken just before insertion of the catheter. The radioactivity in the 2-hr sample was assigned a value of 100% and the radioactivity in subsequent samples was expressed as a percentage of the 2-hr value. Platelet survival was calculated using Murphy's gamma function.

Drug Administration
Heparin. Heparin (150 U/kg) was given intravenously immediately before the insertion of the aortic catheter. Half the initial dose was repeated every 30 min for 2 hr.
Aspirin. Aspirin was given orally through a permanent pharyngeal catheter. The animals were preanesthetized with Ketamine (Ketaset, Rogar/STB, Division of BTA Products, London, Ontario) and the extent of platelet aggregation measured 3 mm after the addition of acid-soluble collagen or sodium arachidonate

Platelet Aggregation Studies
Samples of blood were taken from the central ear artery with a plastic syringe directly into trisodium citrate anticoagulant (3.8%, 9 volumes of whole blood to 1 volume of anticoagulant). The blood was centrifuged at room temperature at 190 g for 15 min, and plateletrich plasma (PRP) obtained. The platelet count was adjusted to 5 x 108/ml with platelet-poor plasma (PPP) prepared from the same blood sample. One milliliter of PRP was stirred at 37°C for 1 min in a Payton Aggregation Module (Payton Associates, Scarborough, Ontario) and the extent of platelet aggregation measured 3 min after the addition of acid-soluble collagen or sodium arachidonate

Measurement of PGI2-Like Activity of Thoracic Aorta
For preparation of samples of aortae for measurement of PGI2-like activity, rabbits were anesthetized with 20-40 mg/kg sodium pentobarbital and given an injection of heparin (300 U/kg) before
they were exsanguinated through a carotid cannula. The thoracic aortae were immediately isolated and flushed in situ with Eagle’s medium (GIBCO Laboratories, Grand Island Biological Co., Grand Island, N.Y.), containing 0.35% bovine serum albumin, 5 mM Hepes buffer, pH 7.45. The aortae were removed from the animals, cleared of adventitia, and cut into 0.5-cm rings. The vessel rings were stored in Eagle’s medium at 37°C for 2 hr.

After 2 hr, each ring was incubated in 150 µg Eagle’s medium containing 50 µM arachidonic acid at 37°C for 3 min; 100 µl of the incubation medium was then added to 1 ml of a suspension of aspirin-treated washed rabbit platelets that had been prelabeled with [14C]-serotonin and resuspended in a medium containing imipramine (10 µM, Ciba-Geigy, Dorval, Quebec). The sample was stirred in a Payton Aggregation module at 37°C. Sixty seconds later, thrombin (0.05 U/ml, an amount of capable of inducing 40%-50% stirred in a Payton Aggregation module at 37°C. Sixty seconds later, thrombin (0.05 U/ml, an amount of capable of inducing 40%-50% release of serotonin and resuspended in a medium containing imipramine. The Tyrode-albumin solution was then discarded and the tubes aspirated, and 3 ml of modified Tyrode solution (calcium and magnesium omitted) containing 10 mg/ml bovine serum albumin (Sigma) was added to each tube and allowed to incubate overnight at 4°C. The Tyrode-albumin solution was then discarded and the tubes washed 3 times with modified Tyrode solution containing 0.1% sodium azide and stored at 4°C until required for an assay. Approximately 2 µg of fibrinogen (fibrin) was added to each polyethylene tube, and the radioactivity in the solution counted in a gamma scintillation counter.

Whole Blood Fibrinolytic Activity

Whole blood fibrinolytic activity was measured using the solid-phase radiometric assay described by Moroz and Gilmore. [125I]-fibrin-coated tubes were prepared using a slight modification of the method described. Human fibrinogen (grade L, AB Kabi, Stockholm, Sweden) was treated with diisopropylfluorophosphate (DFP, Sigma) to inactivate any serine proteases and purified by passage through a DEAE-cellulose column. Fibrinogen was labeled with [125I] (Amersham/Searle) using iodine monochloride, and 0.5 ml was added to each polyethylene tube (12 × 75 mm). The tubes were shaken for 3 hr at 37°C after which the [125I]-fibrinogen solution was aspirated, and 3 ml of modified Tyrode solution (calcium and magnesium omitted) containing 10 mg/ml bovine serum albumin (Sigma) was added to each tube and allowed to incubate overnight at 4°C. The Tyrode-albumin solution was then discarded and the tubes washed 3 times with modified Tyrode solution. One milliliter of Tyrode solution containing 2 mM CaCl2 and 2 U of plasmin-free human thrombin (grade I, Sigma) were added and incubated at 37°C for 15 min. The thrombin solution was then discarded and the tubes rinsed 3 times with modified Tyrode solution before they were filled with modified Tyrode solution containing 0.1% sodium azide and stored at 4°C until required for an assay. Approximately 2 µg of fibrinogen (fibrin) became associated with the wall of the tube.

The assay for whole blood fibrinolytic activity is based on release of radioactivity from the [125I]-fibrin bound to the polyethylene tubes. One milliliter of whole blood, collected in heparin (10 U/ml), was added to each tube and allowed to incubate at 37°C for 60 min, after which an excess of modified Tyrode solution (2 ml) containing 10 U/ml heparin was added, the tube contents transferred to a polyethylene tube, and the radioactivity in the solution counted in a gamma scintillation counter.

Prothrombin Time and Thrombin Time

These clotting assays were performed on plasma from blood samples collected in trisodium citrate anticoagulant (3.8%, 9 parts blood and 1 part anticoagulant) according to standard techniques. The thromboplastin reagent for prothrombin time determinations was purchased from the National Reference Laboratory for Anticoagulant Reagents and Control, Withington Hospital, Manchester, U.K. For thrombin time determinations, Pentex bovine thrombin was used (Miles Laboratories Inc., Elkhart, Ind.).

Plasma Salicylate Levels

Plasma salicylate levels were determined by the method described by Trinder.

Statistical Methods

Because of the multiple comparisons used in these experiments and because the studies were designed to determine the effects of high doses of aspirin, the statistical analysis for platelet survival and thrombus weights was performed using orthogonal contrasts.

RESULTS

In preliminary experiments to determine whether thrombin might be an important factor in the initiation and growth of the thrombi that formed on and around the catheter, we examined the effects of administering heparin during the early period following introduction of the catheter on the extent of thrombus formation. High doses of heparin (150 U/kg initially followed by 75 U/kg at half-hour intervals over a 2-hr period) produced about a 90% reduction in the amount of thrombus associated with the catheter at 2 hr (thrombus weight: heparin-treated, 0.90 ± 0.2 mg; control, 9.3 ± 4.05 mg; mean ± SEM for 3 rabbits in each group). Thus, thrombin generation contributes to thrombus formation caused by the indwelling aortic catheters.

The thrombi that formed on and around the indwelling aortic catheters reached maximum size by about 24 hr and showed little increase in size thereafter. Histologic examination of thrombi at either 3 hr or 5 or 6 days showed them to contain platelets and substantial amounts of fibrin, red blood cells, and white cells (Fig. 1, A and B). The proportion of fibrin appeared to be greater in the older thrombi.

Administration of either high or low doses of aspirin did not significantly affect thrombus formation in association with the catheter and vessel wall in the first 3 hr (Table 1), although the mean thrombus weight tended to be greater in the animals given the highest dose of aspirin. However, at 5 days, none of the doses of aspirin enhanced thrombosis; with the very high dose (200 mg/kg every 8 hr), there was a tendency for the amount of thrombus to be reduced, although under the conditions of these experiments the mean thrombus weight was not significantly different from the values for the animals given the lower doses of aspirin or from controls.

Platelet survival was significantly shortened in rabbits with indwelling aortic catheters (sham-operated...
Fig. 1. Histologic appearance of (A) a fresh (3 hr) thrombus contains platelet aggregates (plt), fibrin (fib), red blood cells (rbc), blood cells, white blood cells, and a few platelet aggregates. Smooth muscle cells (smc) have begun to enter the thrombus. (A) MSB stain; and (B) a thrombus formed over a 6-day period. The 3-hr thrombus and white cells (wbc). The 6-day thrombus contains fibrin, some red blood cells (wbc). The 6-day thrombus contains fibrin, some red

control, 71.6 ± 3.8 hr, n = 13; indwelling aortic catheter, 42.6 ± 3.3, n = 15). None of the doses of aspirin, including the very high dose that tended to inhibit thrombus formation, significantly prolonged shortened platelet survival; there were 13 or 14 animals used for each of the 4 doses of aspirin used in Table 1. In the sham-operated animals, platelet survival was significantly longer than for the controls only in animals treated with the lowest dose of aspirin (10 mg/kg) (platelet survival 96.2 ± 5.1 hr, p < 0.005).

For all aspirin regimens, platelets in citrated plasma prepared from samples of blood taken 1 hr after the first dose on the second day (25 hr) did not aggregate in response to sodium arachidonate or acid-soluble collagen. Platelets taken just before the second dose on the second day (32 hr) from animals given aspirin three times daily, also did not respond to these aggregating agents. Platelets taken just before the third dose at 48 hr from animals given a daily dose of 10 or 100 mg/kg had partially recovered their ability to aggregate in response to sodium arachidonate or acid-soluble collagen.

### Table 1. Effect of Oral Aspirin on Thrombus Weight in Rabbits With Indwelling Aortic Catheters

<table>
<thead>
<tr>
<th>Group</th>
<th>Aspirin* (mg/kg)</th>
<th>Thrombus Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 3 hr†</td>
<td>At 5 Days</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>8.1 ± 1.9 (9)</td>
</tr>
<tr>
<td>B</td>
<td>10 daily</td>
<td>6.0 ± 1.5 (8)</td>
</tr>
<tr>
<td>C</td>
<td>100 daily</td>
<td>10.4 ± 2.2 (8)</td>
</tr>
<tr>
<td>D</td>
<td>50 t.i.d.</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>200 t.i.d.</td>
<td>12.4 ± 2.5 (9)</td>
</tr>
</tbody>
</table>

Mean values ± SEM. The number of animals in each experiment is indicated in parenthesis.

The significance of the differences among the thrombus weights at 5 days for the 5 groups was calculated using orthogonal contrasts. Both thrombus weights and the logarithms of the thrombus weights were used in these calculations. The groups compared were: A vs B, C, D, E — NS; E vs B, C, D — NS; B vs C, D — NS; C vs D — NS.

*Aspirin was administered orally by pharyngeal tube 2 hr before the introduction of the aortic catheter and thereafter at the times indicated.

†Thrombus weight following a single dose of aspirin.

### Table 2. Effect of Oral Aspirin on PG12-Like Activity Produced From Added Sodium Arachidonate by Rabbit Aorta Samples

<table>
<thead>
<tr>
<th>Time After First Dose of Aspirin</th>
<th>10</th>
<th>100</th>
<th>50</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily</td>
<td>Daily</td>
<td>t.i.d.</td>
<td>t.i.d.</td>
</tr>
<tr>
<td>25 hr</td>
<td>93.8 ± 5.4</td>
<td>26.3 ± 6.4</td>
<td>25.6 ± 7.6</td>
<td>10.4 ± 4.9</td>
</tr>
<tr>
<td>32 hr</td>
<td>—</td>
<td>—</td>
<td>54.6 ± 7.5</td>
<td>52.1 ± 8.8</td>
</tr>
<tr>
<td>48 hr</td>
<td>93.5 ± 3.6</td>
<td>65.9 ± 8.5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean values ± SEM of 6 samples of aorta from each of 3 animals in each group.

*See Materials and Methods.
The formation of PG\(_1\) like activity by rabbit aortae was not inhibited by a dose of 10 mg/kg aspirin daily (Table 2). Aspirin, at a dose of 100 mg/kg daily, reduced PG\(_1\) like activity to 26% of the control 1 hr after the second dose of aspirin (25 hr), but at 48 hr, the PG\(_1\) like activity of the aorta had recovered to 66% of the control. When aspirin was given 3 times daily (50 mg/kg or 200 mg/kg), the inhibitory effect on PG\(_1\) like activity observed at 25 hr (1 hr after the third dose of aspirin) had returned to approximately one-half of the control values by 32 hr (Table 2). Several groups have also examined the effect of aspirin treatment of rabbits, rats, and humans on PG\(_1\) production by the aorta and found that these vessels regained their ability to produce PG\(_1\) after 24–48 hr.\(^3,4,12,33,34\)

We next examined whether sodium salicylate at a molar concentration equal to that of the 200 mg/kg aspirin would influence the extent of thrombus formation. This dose of sodium salicylate significantly reduced thrombus weight but had no effect on platelet survival in rabbits with indwelling aortic catheters (Table 3). Sodium salicylate at this dose had no effect on thrombus weight at 3 hr; sodium salicylate, 8.8 ± 2.5 mg; placebo, 8.1 ± 1.9 mg.

One of the reported effects of high concentrations of sodium salicylate is an increase of whole blood fibrinolytic activity.\(^33\) High concentrations of sodium salicylate or aspirin (1.55–4.1 mM) added to heparinized rabbit blood increased whole blood fibrinolytic activity by 30%–40%. The effect of intravenous administration of sodium salicylate or aspirin on whole blood fibrinolytic activity over a 2-hr period is shown in Table 4. Aspirin or sodium salicylate significantly increased whole blood fibrinolytic activity, and there was no significant difference between the extent of the increase induced by these drugs. Fibrinolytic activity was also increased in the blood of rabbits given aspirin or sodium salicylate over 3 days (placebo, 9.3% before and 8.5% after 3 days; aspirin, 200 mg t.i.d., 8.8% before and 14.6% after 3 days; sodium salicylate, 177.2 mg t.i.d., 8.08% before and 11.7% after 3 days). The correlation coefficient between plasma salicylate levels and whole blood fibrinolytic activity of rabbits given different oral doses of aspirin or sodium salicylate was \(r = 0.55\) (\(p < 0.001, n = 36\)).

The histologic appearance of 5-day-old thrombi from animals given high doses of aspirin or sodium salicylate showed a loosening of fibrin in the thrombus with clear areas around the leukocytes (Fig. 2, A, B, and C).

High doses of aspirin or sodium salicylate can inhibit the synthesis of the vitamin K-dependent clotting factors.\(^30,41\) In the present experiments, the administration of high doses of aspirin or sodium salicylate for three days significantly prolonged the prothrombin time (Table 5). The thrombin time was not prolonged.

### DISCUSSION

In the experiments reported in this article, only the very high dose of aspirin tended to inhibit thrombosis induced by continuous vessel injury; lower concentrations of aspirin that effectively inhibited the response

### Table 3. Effect of Oral Sodium Salicylate on Platelet Survival and Thrombus Weight in Rabbits With Indwelling Aortic Catheters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Platelet Survival (hr)</th>
<th>Thrombus Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>58.7 ± 3.7</td>
<td>25.6 ± 4.7</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>56.7 ± 4.9†</td>
<td>7.2 ± 1.6*</td>
</tr>
<tr>
<td>(177.2 mg/kg t.i.d.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SEM. There were 9 animals in each experiment. Sodium salicylate was given orally by pharyngeal tube 2 hr before the introduction of the aortic catheter and 3 times daily for 5 days. \*p < 0.005 calculated using Student's t test.

### Table 4. Effect of Intravenous Injection of Aspirin or Sodium Salicylate on Rabbit Whole Blood Fibrinolytic Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Percent Radioactivity Released From (^{125})Fibrin-Coated Tubes* Time after treatment (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6</td>
<td>6.4 ± 1.0 0 6.2 ± 0.9 30 5.4 ± 0.6 60 6.2 ± 0.9 120</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg/kg</td>
<td>6.4 ± 0.6 30 8.4 ± 0.9† 60 9.0 ± 9.6† 120 5.5 ± 0.4</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>177.2 mg/kg</td>
<td>6</td>
</tr>
</tbody>
</table>

Mean values ± SEM.

The data were analyzed using Student's t test; the values for blood from animals that received the drugs were compared with the corresponding values for blood from animals that received placebo.

*See Materials and Methods

Significance of difference from corresponding placebo:

†p < 0.001
‡p < 0.01
§p < 0.05
Table 5. Prothrombin Time of Rabbit Plasma After 3 Days of Treatment With Aspirin or Sodium Salicylate

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of Animals</th>
<th>Prothrombin Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4</td>
<td>11.0 ± 0.1</td>
</tr>
<tr>
<td>Aspirin 10 mg/kg/daily</td>
<td>4</td>
<td>10.9 ± 0.1</td>
</tr>
<tr>
<td>Aspirin 100 mg/kg/daily</td>
<td>4</td>
<td>10.9 ± 0.09</td>
</tr>
<tr>
<td>Aspirin 50 mg/kg/t.i.d.</td>
<td>4</td>
<td>11.2 ± 0.1</td>
</tr>
<tr>
<td>Aspirin 200 mg/kg/t.i.d.</td>
<td>4</td>
<td>100.2 ± 10.95*</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>4</td>
<td>82.4 ± 24.6†</td>
</tr>
</tbody>
</table>

Mean ± SEM

The values for blood from the animals that received the drugs were compared with the corresponding values for blood from animals that received placebo.

*Significance of difference from placebo p < 0.001.
†Significance of difference from placebo p < 0.025.

of platelets to stimuli such as sodium arachidonate or collagen did not affect thrombosis. Zimmerman et al.42 found that there was a tendency for a high dose of aspirin (100 mg/kg) to enhance thrombosis in rabbits, whereas with prolonged treatment, the thrombi were smaller than those that formed in animals given 10 mg/kg.

Aspirin is a weak inhibitor of thrombin-induced platelet aggregation and release reaction.10 The failure of doses of aspirin that inhibited platelet function to prevent thrombosis could be attributable to the observation that thrombin seems to play a large part in the initiation and growth of thrombi under these experimental conditions, since the amount of thrombus that formed around an indwelling aortic catheter in the first
few hours could be greatly reduced by treating the animals with heparin. In addition, the doses of aspirin or sodium salicylate that inhibited thrombus formation over a 5-day period increased the one-stage prothrombin time. Although the high doses of aspirin did not produce a significant inhibition of thrombus formation, the lower mean value in this group is compatible with the reduction in the amount of thrombus material caused by the administration of sodium salicylate.

Since PG1 is a potent inhibitor of platelet aggregation, there has been some concern that drugs that inhibit cyclo-oxygenase, such as indomethacin or aspirin, and thus block PG1 production by vessel walls may be thrombogenic. In the first few hours following insertion of the catheter, the high doses of aspirin that inhibited PG1 formation tended to promote thrombus formation, although not to a statistically significant degree. This is similar to the thrombogenic effect of high doses of aspirin that was observed by Kelton et al. and Buchanan et al. in experiments with injured jugular veins and carotid arteries in rabbits. In both these types of experiments, thrombin generation would have played a major part in the formation of the thrombi. Both thrombin and mechanical injury stimulate PG1 production by the endothelium. PG1 can inhibit the enhancing effect of activated platelets on thrombin generation and inhibit platelet aggregation. Thus, in circumstances in which platelet aggregates may be important in enhancing thrombin generation, such as during the early stages of thrombus formation, inhibition of PG1 production may be thrombogenic. However, inhibition of PG1 formation did not enhance the size of the thrombus that was present after 5 days of continuous injury. The failure of high doses of aspirin to produce a significant inhibition of thrombosis (whereas an equivalent dose of sodium salicylate was inhibitory) could be due to aspirin inhibition of PG1 formation.

The effectiveness of aspirin as an inhibitor of experimental thrombosis is controversial, since there is wide variation in the results obtained when it is used in different experimental circumstances; sometimes it is inhibitory and sometimes it is not. It may be that much of the variation in the ability of aspirin to inhibit experimental arterial thrombosis is related to whether or not the response to vessel injury involves a largely thrombin-dependent reaction.

In the present experiments, the tendency for high doses of aspirin or sodium salicylate to inhibit thrombus formation secondary to chronic vessel injury appeared to be related to a prolongation of the one-stage prothrombin time as well as to activation of whole blood fibrinolytic activity, which of these pathways was most important in reducing the amount of thrombus present at 5 days is not known. It is probable that these mechanisms were complementary, and the morphological observation of an apparent increase in the digestion of fibrin around the white cells in the thrombi indicates that there was increased fibrinolytic activity in the thrombi of animals treated with the high doses of aspirin or sodium salicylate. This is in keeping with Moroz' observation that high doses of aspirin and sodium salicylate increases whole blood fibrinolytic activity through an effect of the protease action of white blood cells. There are reports that doses of aspirin as low as 2–3 g/day in man can prolong the prothrombin time; these doses of aspirin are also reported to increase whole blood fibrinolytic activity. It may be that the beneficial effect of high doses of aspirin (3 g/day) in preventing venous thrombosis and pulmonary embolism is not just due to the effect of aspirin on platelets, but could also be related to the effect of aspirin on blood coagulation and fibrinolysis.

In man, the combination of aspirin and oral anticoagulants has been reported to be more effective in inhibiting thromboembolic complications than oral anticoagulants alone.

The results from these experiments show that aspirin and sodium salicylate did not prolong the shortened platelet survival caused by the indwelling aortic catheter even at concentrations that reduced thrombus formation. Thus, under these experimental conditions, changes in the extent of thrombosis are not related to changes in platelet survival. Salicylate-containing drugs can decrease thrombosis without affecting platelet survival. The lack of effect of aspirin on platelet survival has also been reported by other investigators.

Although it is difficult to apply results obtained from experiments in animals directly to human problems, it is clear from these studies that aspirin in doses that only inhibit platelet function is not a satisfactory inhibitor of arterial thrombosis when there is a significant fibrin component. Since it is likely that multiple pathways are involved in thrombus formation in diseased human arteries, it is not surprising that aspirin has not been found to be a strongly effective inhibitor of the thromboembolic clinical complications of arterial disease in man. In very high doses, aspirin may inhibit thrombosis when thrombin makes a large contribution, but this effect is not mediated through inhibition of platelet function, since sodium salicylate, which has little effect on platelet function, is more effective than aspirin. It may be that in studies in which aspirin has been reported to reduce the incidence of venous thrombosis and pulmonary embolism in man, the dosage has been high enough, and the drug has been given over a sufficiently long period of time to affect the coagulation system and whole blood fibrinolytic activity.
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Effect of aspirin and sodium salicylate on thrombosis, fibrinolysis, prothrombin time, and platelet survival in rabbits with indwelling aortic catheters

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