Br. J. Pharmac. (1982), **77**, 029–038

FIBRIN, RED CELL AND PLATELET INTERACTIONS IN AN EXPERIMENTAL MODEL OF THROMBOSIS

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1 Insertion of a cotton thread into an arteriovenous shunt of an anaesthetized rat causes an increase in the weight of the thread due to deposition of thrombus.

2 The thrombus formed was of the venous (red) type, being sensitive to heparin, yet possessed important characteristics of an arterial thrombus, in that it was dependent on platelets and on rate of blood flow.

3 Thromboxane synthetase inhibitors had no effect on thrombus deposition.

4 Cyclo-oxygenase inhibitors did not significantly depress thrombus formation at doses which inhibited platelet function *ex vivo*.

5 Compounds which can modify the release or action of adenosine 5'-diphosphate partly inhibited thrombus formation.

6 A depression in clotting factor levels induced by sodium warfarin led to a highly significant reduction in thrombus formation at doses which caused a prolongation of prothrombin clotting time.

Introduction

Thrombus formation involves the vessel wall, cellular components of the blood, the coagulation system as well as rheological factors. Numerous methods are available to produce experimental thrombosis *in vivo* (Philp, 1979), however, the specific physiological mechanisms involved in thrombus formation in individual models are not always clear, with many models exhibiting a typical red (venous) as opposed to white (arterial) thrombi.

Recently, models have been described by Ashida, Sakuma & Abiko (1980) who used a vascular shunt in rats to produce occlusive thrombi, and Kumada, Ishihara, Ogawa & Abiko (1980) who induced thrombus formation in rats by the insertion of a steel wire into the vena cava. Seuter, Busse, Meng, Hoffmeister, Möller & Horstmann (1979) has described a model in which the vessels of rats were chilled to cause occlusive thrombi. Umetsu & Sanai (1978) established an extracorporeal shunt into which a silk thread was placed with subsequent thrombus formation. All of these models appeared to produce typical venous thrombi containing a large proportion of red cells and fibrin. It is the latter model which we have chosen to study in order to find out which pharmacological mediators are involved in the formation of thrombus attached to the thread.

A preliminary account of this work has appeared (Smith & White, 1981).

Methods

Thrombus formation in vivo

The method of Umetsu & Sanai (1978) was modified. Male Cob Wistar rats (450–500 g) were anaesthetized with sodium pentobarbitone (40 mg/kg i.p.) and an incision made over the trachea. The right jugular vein and left carotid artery were exposed and the two ends of the extracorporeal shunt were inserted into them. The shunt consisted of two 12 cm lengths of polyethylene tubing (0.81 mm and 0.58 mm internal diameter, respectively) connected by 5 mm silicone rubber plugs to a 6 cm length of polyvinyl tubing (3 mm internal diameter). A 6 cm length of cotton thread was secured between the two plugs so that it remained longitudinally orientated in the blood flow through the cannula. Before cannulation the tubing was treated with a siliconising fluid ('Repelcote'), allowed to dry and then filled with heparin (50 iu/ml). The shunt was left in place for 15 min after the extracorporeal circulation was started. The flow was then stopped and the thread removed. A second thrombus was then obtained in each animal by replacing the centre tube (3 mm internal diameter) with a new tube and thread containing 0.9% w/v sodium chloride solution (saline). Heparin (0.2 ml 25 iu/ml) was injected into the venous side of the shunt prior to re-starting blood flow. Again flow was maintained for 15 min. There was no

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statistical difference in weight between the first and second thrombus.

The animals were kept at 37°C with a heating lamp controlled by a thermistor probe.

An electromagnetic flow meter (Narco Biosystems Inc.) was placed around the carotid artery to monitor blood flow during the experiment.

Gross thrombus size was defined as the wet weight of the thread (mg), removed from the shunt at the termination of the experiment. Control thrombus weight used in the calculation of data includes the wet weight of the thread which contributes 20 + 2% of the total thrombus weight.

In some experiments the various components of the thrombus were labelled with an appropriate radiolabel and the rate of their disposition into the thrombus recorded.

The effect of blood flow on thrombus formation in vitro

Blood was obtained from rats which were anaesthetized with sodium pentobarbitone (40 mg/kg). The end of a 6 cm length polyethylene tubing (0.81 mm i.d.) was inserted into the carotid artery, and heparin, either 35 iu/kg or 50 iu/kg was injected and within 1 min the blood was withdrawn into a 20 ml syringe, via the cannula. The blood was stored in a reservoir at 37°C and pumped through a shunt, as described above, at differing flow rates. The threads were examined after 15 min.

Light microscopical examination of the thrombus

Threads were removed from the extracorporeal shunt after 15 min *in vivo* and placed in fresh fixative for 4 h at room temperature. The fixative contained 2.5% glutaraldehyde plus 4% formaldehyde (freshly prepared from paraformaldehyde) 2 mM $CaCl_2$ in 0.1 M cacodylate buffer pH 7.2. The thread was then transferred to 0.1 M cacodylate buffer and either processed immediately or kept at 4°C.

The thread was cut into lengths and post fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h at 4°C. The pieces were then washed in 0.1 M cacodylate buffer before being dehydrated through ascending concentrations of ethanol and embedded in an Epon-Araldite resin.

From each thread sample embedded in Epon-Araldite 1 μm–2 μm sections were cut in both transverse and longitudinal planes on an LKB Ultratome III. The sections were heat-fixed on to a glass microscope slide, stained with 0.1% toluidine blue in 0.1% borax buffer and mounted in Epon-Araldite resin.

Electron microscopical examination of the thrombus

Sections (silver-gold) were cut using an LKB Ultratome III with a diamond knife and placed on Athene 200 copper grids. Sections were then stained with 5% uranyl acetate and finally Reynold's lead citrate before being examined under an AEI 6B electron microscope.

Preparation of ^{51}Cr-labelled platelets

Blood (90 ml) from 9 rats was gently stirred into 9.0 ml of 3.8% trisodium citrate. The anticoagulated blood was centrifuged at 190 *g* for 30 min at 4°C to obtain platelet-rich plasma (PRP). The PRP was removed and centrifuged at 400 *g* for 5 min at 4°C to remove remaining red cells. The PRP was centrifuged at 700 *g* for 20 min at 4°C to obtain the first harvest of platelets. The supernatant was centrifuged again to obtain a second harvest, both pellets being resuspended in platelet-poor plasma (PPP) to a total volume of 9 ml. ^{51}Cr-sodium chromate (900 μCi) was added and the platelet concentrate allowed to stand at room temperature for 1 h. The labelled platelet suspension was then diluted through the addition of calcium-free Tyrode solution (45 ml) and the platelets were harvested twice as before. The platelets were finally resuspended for injection in 9.0 ml PPP.

A sample (0.1 ml) of the labelled platelet suspension was centrifuged at 450 *g* for 5 min at room temperature. Providing the number of counts in the supernatant were less than 5% of the total, the rest of the labelled platelet suspensions were injected intravenously into rats (0.1 ml/100 g body weight). The rats were left for 24 h before an extracorporeal circulation was established.

Preparation of ^{51}Cr-labelled red cells

Blood (50 ml) was anticoagulated as described above. The blood was centrifuged at 190 *g* for 20 min at 4°C to obtain PRP. The RBC's were isolated, resuspended to the original volume with isotonic saline and centrifuged at 250 *g* for 20 min at 4°C.

^{51}Cr-sodium chromate (700 μCi) was added to the red cell suspension and left for 1 h at room temperature. The supernatant was removed and the red cells resuspended in saline and harvested again. The labelled cells were injected intravenously into rats (0.1 ml/100 g body weight).

Administration of ^{125}I-fibrinogen

Rats were anaesthetized with ether and 0.5 μCi I^{125} human fibrinogen was injected intravenously. The extracorporeal shunts were inserted 10 min later.

Measurement of the radioactivity

In all three radiolabelling techniques, the thread was

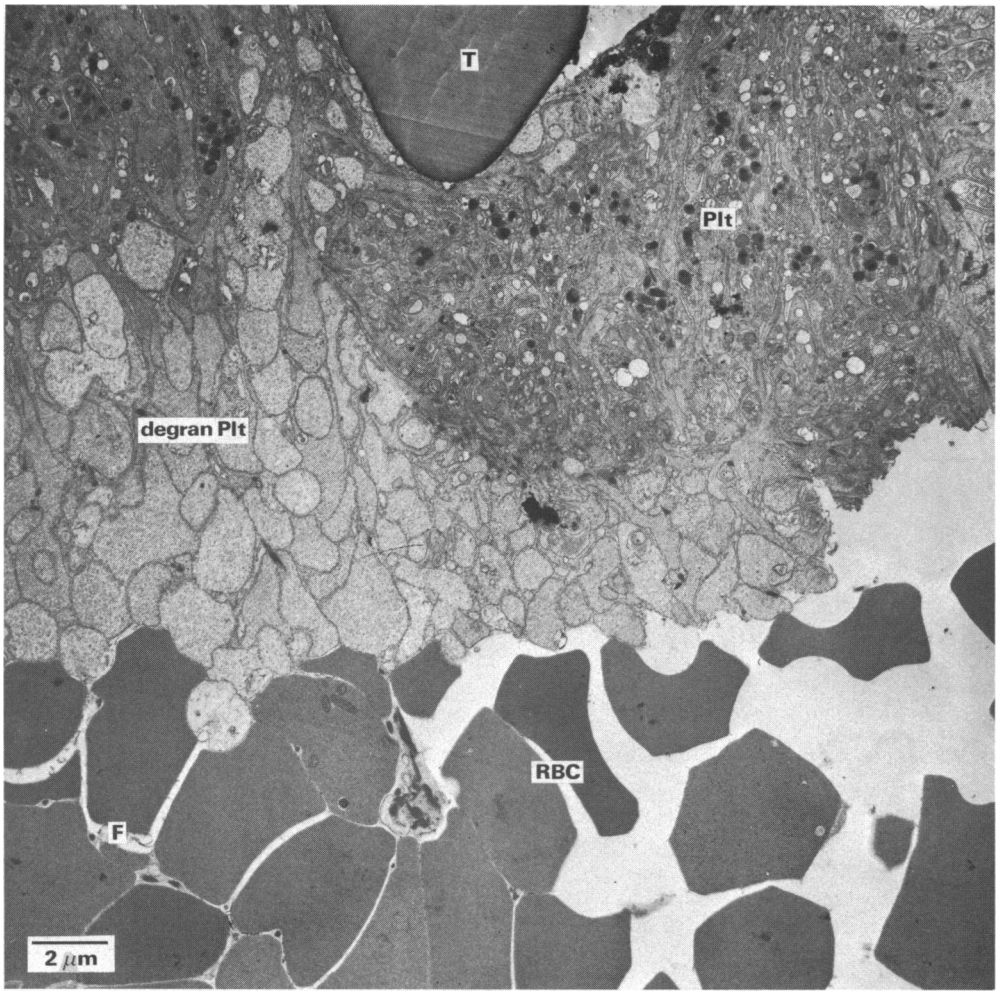

Figure 1(a) Transmission electronmicrograph (magnification × 7500) of the thrombus attached to the thread after 15 min exposure to flowing blood *in vivo*. T = strand of cotton thread; degran Plt = degranulated platelets; Plt = platelets with granules mostly intact; RBC = red blood cells; F = fibrin strands.

removed from the shunt and counted in a Beckman Biogamma Counter. A whole blood sample (0.2 ml) was also taken for counting.

The results were expressed as a ratio of

$$\frac{\text{Radioactivity on thread}}{\text{Radioactivity in blood}} \times 100$$

Reduction in platelet count

Platelet counts were reduced by treatment with rabbit anti-rat platelet antiserum. The antiserum was produced in rabbits by the injection of rat platelet lysate.

Rat platelets were isolated by the double harvesting technique described above for the isolation of ^{51}Cr-labelled rat platelets. Following the second harvest, the platelets were resuspended in saline and lysed by freezing and thawing three times.

Rabbits were immunized with 4 × 1 ml subcutaneous injection of this material suspended in Freund's Complete Adjuvant. Two weeks later, two booster injections were given subcutaneously. After a further 5 weeks the animals were bled, serum was prepared, pooled and divided into 5 ml aliquots, and stored at − 20°C.

For platelet depletion experiments rats received 0.1 ml/100 g body weight of the antiserum, intravenously 24 and 48 h before the experiment. In some experiments, neuraminidase (Sigma) was used to

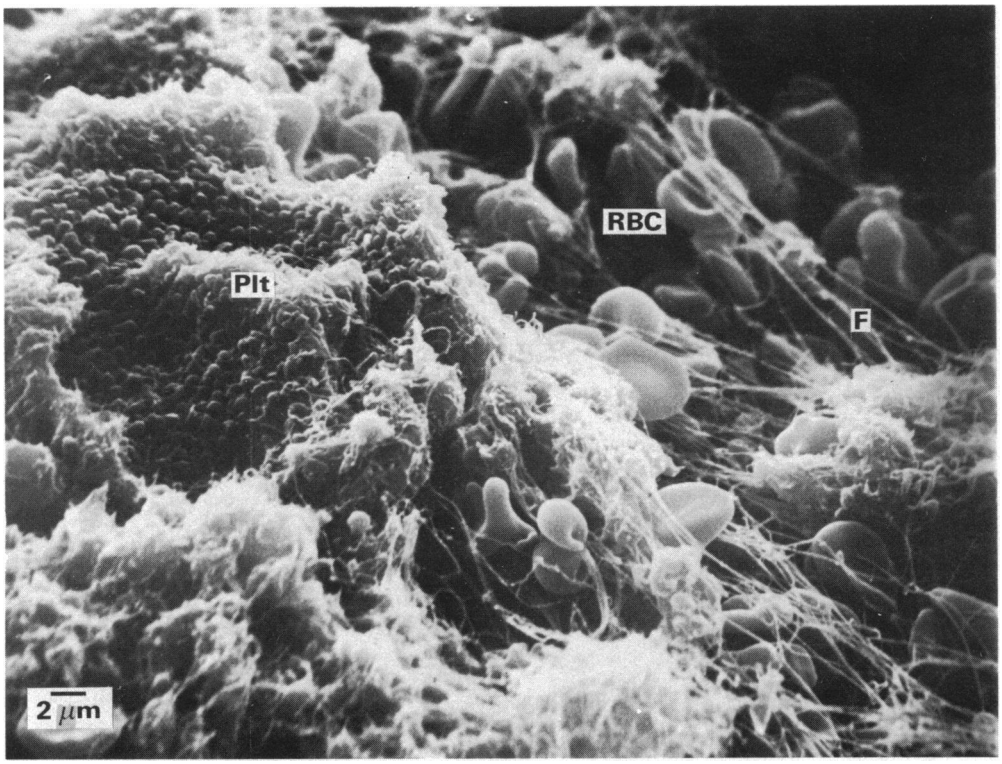

Figure 1(b) Scanning electronmicrograph (magnification × 3000) of the thrombus attached to the thread after 15 min exposure to flowing blood *in vivo*; Plt = platelet aggregate; RBC = red blood cell; F = fibrin strands.

reduce the platelet count. Neuraminidase (4 u/kg) was administered intravenously 72, 48 and 24 h before the experiment.

Platelet aggregation ex vivo *induced by arachidonic acid*

Blood (12 ml) was taken from rats by cardiac puncture under ether anaesthesia into a syringe containing 3.8% trisodium citrate (1.2 ml).

Platelet rich plasma (PRP) was prepared by centrifugation of the blood at 250 *g* for 20 min. Platelet aggregation was measured in a Payton dual channel aggregometer at 37°C. The PRP was stirred at 900 rev/min. Samples were allowed to warm to 37°C for 1 min before adding 10 μl 0.1 M $CaCl_2$ and left for a further minute before adding 2–20 μl of the sodium arachidonate (10 mg/ml arachidonate in 0.1 M Na_2CO_3, stored under nitrogen). The primary rate of aggregation was measured and the log concentration-response curves were plotted for each dose of cyclo-oxygenase inhibitor using at least six different concentrations of arachidonate. The concentration of arachidonate which results in 50% of the maximal aggregation rate (ED_{50}) was calculated. (Inhibition results in an increase in the ED_{50}).

Prothrombin times

Prothrombin time determinations were carried out using the reagents and methods of Dade Diagnostics Inc., Miami, U.S.A. Clotting times were measured in a coagulometer (Burkhead, Uxbridge).

Compounds

Aspirin (BDH and sulphinpyrazone (Ciba-Geigy) were suspended in saline, dissolved by the addition of 1 molar equivalent of NaOH and adjusted to pH 7.4. The sulphide metabolite of sulphinpyrazone, G 25671 required suspension in warm (50°C) saline and was dissolved by the addition of 1.2 molar equivalents of NaOH (final pH approximately 10). Indomethacin (Merck, Sharp & Dohme) was suspended in ethanol (final concentration 50 mg/ml) and dissolved by the addition of 1 molar equivalent of NaOH, and diluted with saline (pH 7.4). Diclofenac (Ciba-Geigy) and bencyclan (Pharmakon A.G.)

were supplied ready for injection. Ticlopidine was suspended in saline and dissolved by the addition of 1 molar equivalent of HCl (pH 5.0). N-(7-carboxyheptyl) imidazole (synthesized by Dr N. Finch, Ciba-Geigy, U.S.A.), imidazole (Sigma), β,γ, methylene ATP (Miles Laboratories) and warfarin sodium (Ward Blenkinsop) were dissolved in saline. Appropriate vehicle controls were used in all experiments.

Statistical analysis

Control and test values were obtained in groups of 5 rats with 10 thrombi per group. Mean data are presented with their standard errors. Student's *t* test was used for all comparisons between test and control groups. *P* values of less than 0.05 were considered statistically significant.

Results

Histological findings in vivo

The flow of blood over a cotton thread results in the formation of a thrombus consisting of significant numbers of red cells, platelets and fibrin (Figure 1a). Light microscopy revealed areas of platelet aggregates in column-like projections from the edge of the thread. These aggregates were surrounded by packed red blood cells. Transmission electron microscopy confirmed that platelets and red cells were the main components of the thrombus, a fibrin network was also seen and it is this which probably entraps the red cells. Transmission electron microscopy also revealed a graded response in the extent of platelet degranulation, where the platelets at the junction with red cells were totally degranulated, compared to platelets closer to the thread whose granules appeared to be retained.

A much clearer view of the fibrin network entrapping the red cells was seen using scanning E.M. (Figure 1b).

Time course of thrombus formation in vivo

The rates of deposition of the components above, namely, platelets, red cells and fibrin were measured by using the appropriate radiolabel (Figure 2).

Platelets labelled with ^{51}Cr accumulated on the thread in a time-dependent manner. A similar rate of accumulation was seen for ^{125}I fibrinogen, and ^{51}Cr red blood cells.

Effect of blood flow on thrombus formation in vitro

An increase in blood flow resulted in an increase in

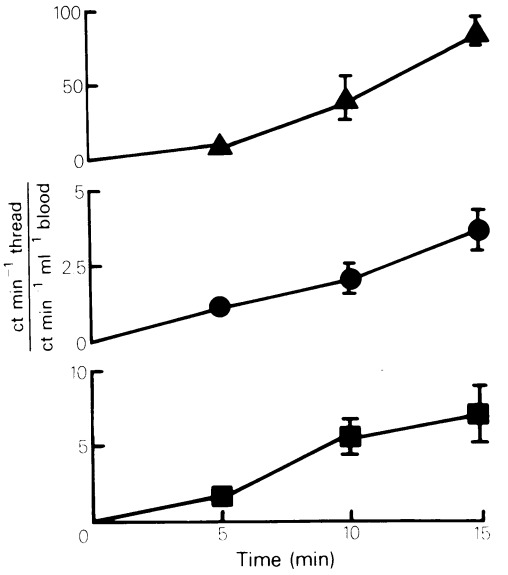

Figure 2 Time course of thrombus formation *in vivo*. Animals were prelabelled with (▲) ^{51}Cr platelets, (●) ^{51}Cr-RBC and (■) I^{125} fibrinogen. The thread was removed at intervals and counted. Each point represents the mean of five experiments; vertical lines show s.e.mean.

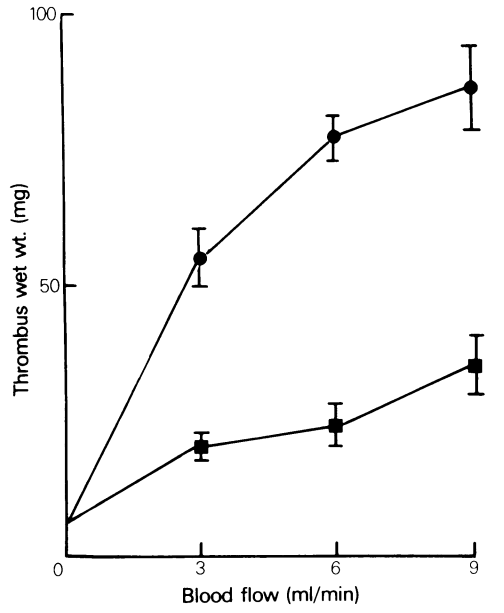

Figure 3 Effect of blood flow on thrombus formation *in vitro*. Blood was obtained from animals given 50 iu/kg heparin i.v. (■) or 35 iu/kg heparin i.v. (●). Using different flow rates, the thread wet weight was determined at time intervals. Each point represents the mean of five experiments; vertical lines show s.e.mean.

the rate of thrombus formation (Figure 3). The thrombus formed was histologically similar to that seen *in vivo*. Blood flow *in vivo* was monitored and was within the range of 3–6 ml/min.

Reduction in the platelet count

The platelet count was reduced to 27 ± 2.2% of control values when rats were pretreated with neuraminidase whilst pre-treatment with rabbit anti-rat platelet antisera reduced the count to 20 ± 9.1% of control values.

In both cases, there was a significant reduction in thrombus formation *in vivo* (Figure 4).

Investigation of possible mediators of thrombus formation

All compounds were administered intravenously 10 min before establishing an extracorporeal shunt.

Effect of thromboxane synthetase inhibitors on thrombus formation (Figure 5) The intravenous administration of imidazole and N-(7-carboxyheptyl) imidazole, failed to reduce thrombus formation significantly when given in doses up to 30 mg/kg (i.v.).

Effect of cyclo-oxygenase inhibitors on thrombus formation (Figure 6) Aspirin did not cause a significant reduction in thrombus formation when tested at doses of 30, 10 and 3 mg/kg (i.v.).

Diclofenac (10 mg/kg) significantly reduced thrombus formation (32.8% ± 10.02% inhibition of control) but not when tested at lower doses of 3 and 1 mg/kg.

Indomethacin (10 mg and 3 mg/kg) caused significant reductions in thrombus formation (24.5 ± 5.1% and 29.02 ± 8.4%).

Effect of sulphinpyrazone and its thioether metabolite (G 25671) on thrombus formation (Figure 7). Sulphinpyrazone (100 mg/kg, i.v.) significantly reduced thrombus formation (45.59 ± 3.7% inhibition of control) but not when tested at the lower dose of 50 mg/kg, (i.v.). The thioether metabolite (G 25671) caused a significant reduction in thrombus formation (28.66 ± 7.02%) when tested at 5 mg/kg (i.v.). Lower doses were ineffective.

Platelet aggregation ex vivo *induced by arachidonic acid (Table 1)* The intravenous administration of cyclo-oxygenase inhibitors to groups of rats ($n = 5$), at doses which did not significantly reduce thrombus formation, resulted in a statistically significant increase in the concentration of sodium arachidonate required to cause an aggregation rate of 50% maximal (ED_{50}).

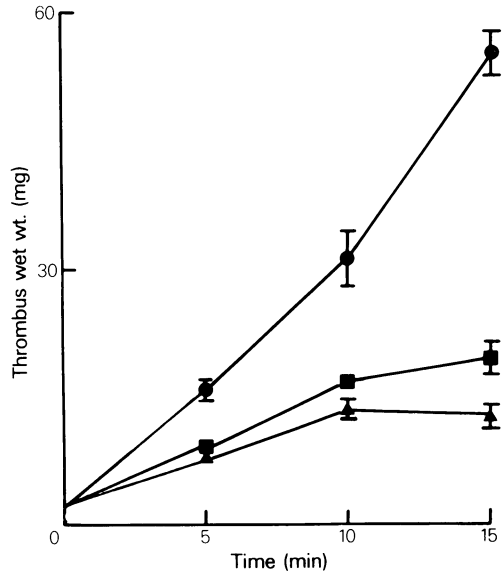

Figure 4 Effect of lowering the platelet count on thrombus formation *in vivo*. (●) Control animals; (■) animals pretreated with neuraminidase (platelet count reduced by 73%); (▲) animals pretreated with rabbit anti-rat platelet antiserum (platelet count reduced by 80%). Each point represents the mean of five experiments; vertical lines show the s.e.mean.

The investigation of the role of ADP in thrombus formation (Figure 8) An infusion of β-γ methylene ATP (200 and 100 μg/min) caused a significant reduction in thrombus formation (51.1 ± 6.2 and 46.02 ± 13.08%) compared to animals receiving an infusion of saline.

Ticlopidine (30 and 10 mg/kg, i.v.) also significantly reduced thrombus formation by 30.05 ± 9.4 and 37.4 ± 4.5%.

Chlorpromazine (10 mg/kg i.v.) significantly reduced thrombus formation (23.9 ± 5.6%) but caused a 15.5 ± 3.4% reduction in blood flow through the shunt. However, chlorpromazine (3 mg/kg i.v.) caused a similar reduction in blood flow (15.75 ± 5.18%) but was found not to alter thrombus formation significantly. Bencyclan (10 mg/kg) caused a 28.76 ± 2.86% reduction in thrombus formation, which was significantly different from the controls.

The investigation of the role of thrombin in thrombus formation Pretreatment of animals with doses of sodium warfarin greater than 0.1 mg/kg (p.o.) caused a significant reduction in thrombus formation. This paralleled a statistically significant prolongation of prothrombin clotting times.

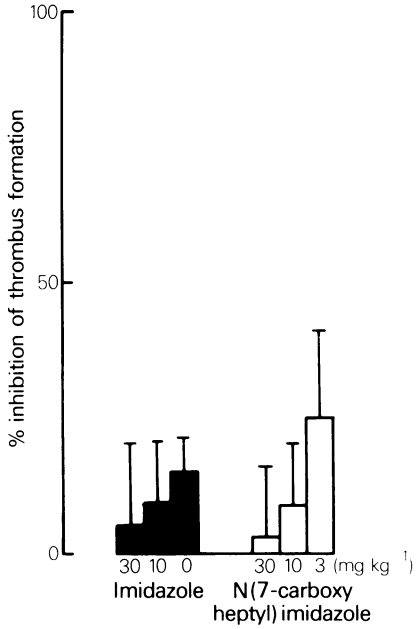

Figure 5 Effect of thromboxane synthetase inhibitors on thrombus formation. Drugs were administered intravenously 10 min prior to establishing an extracorporeal circulation. Solid columns: imidazole; open columns: N(7-carboxyheptyl) imidazole. Each column represents the mean for seven animals per group; (except N(7-carboxyheptyl) imidazole 3 mg/kg i.v. where $n=14$). Vertical lines show s.e.mean. *denotes significant difference when compared to control values ($P<0.05$).

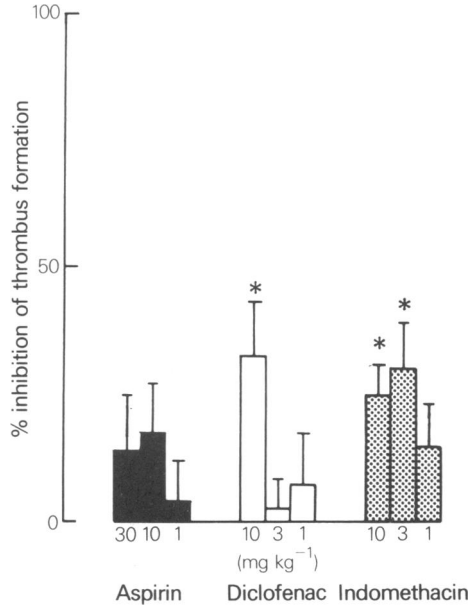

Figure 6 Effect of cyclo-oxygenase inhibitors on thrombus formation *in vivo*. Drugs were administered intravenously 10 min prior to establishing an extracorporeal circulation. Solid columns: aspirin; open columns: diclofenac; stippled columns: indomethacin. Each column represents the mean for seven animals per group; (except aspirin 30 mg kg^{-1} and indomethacin 1 mg kg^{-1} where $n=14$). Vertical lines show s.e.mean. *denotes significant difference when compared to control values ($P<0.05$).

Discussion

The insertion of a cotton thread into an extracorporeal shunt resulted in the formation, on the thread, of a thrombus composed of red cells, platelets and fibrin.

The components of the thrombus built up on the thread at similar rates as measured by a radiolabelling technique (Figure 2).

An examination of the effect of blood flow in this model showed that an increase in flow resulted in an

Table 1 Effect of cyclo-oxygenase inhibitors on platelet aggregation *ex vivo* induced by arachidonic acid

Treatment	(n)	*Dose* (mg/kg)	ED_{50} (μM)	P *value*
Control	(18)	—	300 ± 23	
Aspirin	(5)	30	1487 ± 121	$P<0.001$
	(5)	10	1402 ± 156	$P<0.001$
	(5)	1	712 ± 91	$P<0.001$
Diclofenac	(5)	10	1390 ± 78	$P<0.001$
	(5)	3	1293 ± 148	$P<0.001$
	(5)	1	860 ± 48	$P<0.001$
Indomethacin	(5)	10	1440 ± 66	$P<0.001$
	(5)	3	1290 ± 140	$P<0.001$
	(5)	1	1208 ± 59	$P<0.001$
Sulphinpyrazone	(5)	100	470 ± 28	$P<0.01$
	(5)	50	497 ± 27	$P<.01$
G25671	(5)	5	440 ± 37	$P<.01$
	(5)	1	360 ± 15	$P<.05$

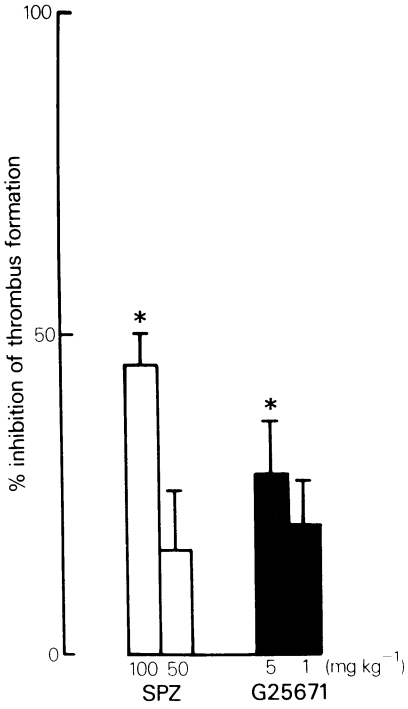

Figure 7 Effect of sulphinpyrazone (SPZ) and its thioether metabolite (G 25671) on thrombus formation. Drugs were administered intravenously 10 min prior to establishing an extracorporeal circulation. Open columns: sulphinpyrazone; solid columns: G 25671. Each column represents the mean for seven animals per group; vertical lines show s.e.mean. *denotes significant difference when compared to controls values ($P<0.05$).

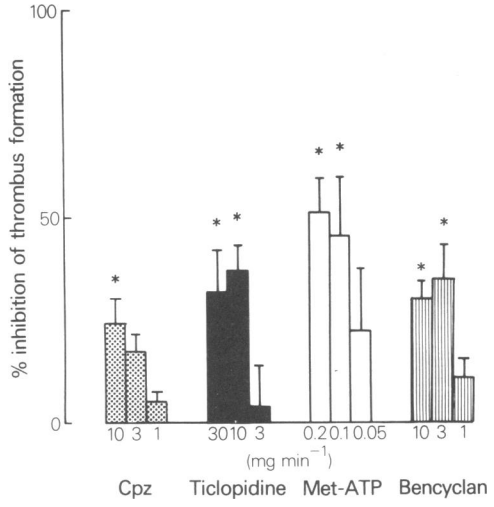

Figure 8 The investigation of the role of ADP in thrombus formation. Drugs were administered intravenously 10 min prior to establishing an extracorporeal circulation, except β-γ-methylene-ATP which was infused for the duration of the experiment. Cpz = chlorpromazine; Met-ATP = β-γ-methyleneadenosine triphosphate. Stippled columns: chlorpromazine; solid columns: ticlopidine; open columns: β-γ-methylene ATP; striped columns: bencyclan. Each column represents the mean of seven animals per group; vertical lines show s.e.mean. *denotes significant difference when compared to control values ($P<0.05$).

increase in thrombus weight, a similar observation to that found by Baumgartner (1973). The thrombus formed *in vitro* was histologically similar to that found *in vivo*.

A reduction in the platelet count, using antisera or neuraminidase pretreatment resulted in a significant reduction in thrombus formation, indicating that the formation of the thrombus was platelet-dependent. This finding, together with the fact that thrombus formation is greater at higher flow rates shows that although the thrombus appears to be a red (venous) thrombus, it has, at least in two important respects, the characteristics of an arterial thrombus.

Investigations into the possible mediators of thrombus formation were carried out using firstly compounds which inhibit the prostaglandin pathway. PGG_2, PGH_2 and thromboxane A_2 are substances which are produced during secondary platelet aggregation (Hamburg, Svensson & Samuelsson, 1975; Smith, Ingermann & Silver, 1976) and inhibition of the cyclo-oxygenase enzyme would prevent the formation of all these substances, and thromboxane synthetase inhibitors the production of thromboxane A_2 (TXA_2), the most potent naturally occuring stimulator of platelet aggregation known (Needleman, Minkes & Raz, 1976). Consequently, we investigated four compounds which are inhibitors of cyclooxygenase, two inhibitors of thromboxane synthetase and the antithrombotic agent, sulphinpyrazone.

Imidazole and N-(7-carboxyheptyl) imidazole are inhibitors of the platelet thromboxane synthetase (Tai & Yuan, 1978; Yoshimoto, Yamamoto & Hayaishi, 1978) and were ineffective against thrombus formation in this model, even at doses which significantly prolong rat tail bleeding time and inhibit platelet TXB_2 production (Butler, Maguire, Smith, Turnbull, Wallis & White, 1981). All the cyclooxygenase inhibitors at doses which were not effective in reducing thrombus formation significantly shifted the dose-response curve to the right when platelet aggregation was induced with sodium arachidonate.

This indicates that the platelet response to prostaglandins was inhibited and it must be through a mechanism other than prostaglandin activity, that these drugs are acting in this model. Inglot & Wolna (1967) reported that non-steroidal anti-inflammatory drugs (NSAIDs) stabilize erthrocyte

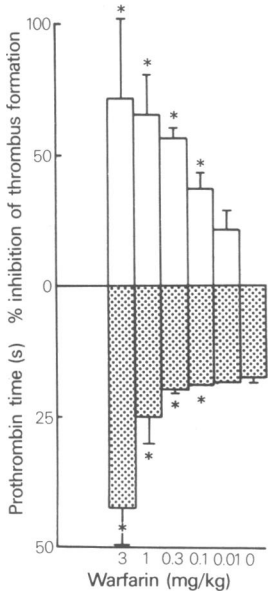

Figure 9 Effect of sodium warfarin on thrombus formation *in vivo* and prothrombin clotting time *ex vivo*. The drug was administered orally 48 h prior to establishing an extracorporeal circulation. Each column represents the mean for five animals per group: vertical lines show s.e.mean. *denotes significant difference when compared to control values ($P<0.05$).

membranes and it is a possibility that the high doses of NSAIDs needed to be effective in this study exerted their activity through inhibition of the release of ADP from red blood cells.

The fact that degranulated platelets are seen in the electron-micrograph in close association with red blood cells, but are not degranulated in other areas suggests that red blood cells are important in this model. It has been suggested by Born & Wehmeier (1979) that red blood cells can leak ADP and participate in this manner in thrombus formation.

This possibility is supported by the fact that thrombus formation in our model is inhibited by the ADP antagonist, β,γ-methylene (Born & Foulkes, 1977; Evans, 1979) and by agents which have been reported to stabilize red cell membranes, viz. bencyclan and chlorpromazine (Kinsella, Smith & Spector, 1978). ADP leakage is however unlikely to have caused the platelet secretion observed since this has never been reported in the rat (Mills, 1970, Dodds, 1978). It is therefore probably only a contributing factor in thrombus formation.

The anti-thrombotic compound ticlopidine is an inhibitor of ADP-induced aggregation in this species (Johnson, Walton, Cotton & Strachan, 1977) but has also been reported to inhibit platelet aggregation induced by other agonists, notably thrombin. The inhibitory action of ticlopidine in our model may reflect this latter activity. This possibility is supported by the fact that heparin and also depletion of clotting factors by sodium warfarin results in markedly reduced thrombus formation. Depletion of clotting factors by sodium warfarin resulted in a much greater inhibition than seen with other drugs. It is also noticeable that large quantities of fibrin are deposited in the thrombus and clearly therefore thrombin formation is an important mediator in cotton thread induced thrombus formation.

Thanks are expressed to Messrs Kemmenoe, Sibbons and Williamson for microscopy and to Drs Butler and Wallis for their invaluable discussions.

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(*Received December 15, 1981.*
Revised May 10, 1982.)