To confirm that the cytotoxic reaction was specific for the H-Y antigen, portions of antiserum to H-Y were absorbed with equal numbers of male and female B6 spleen cells (four volumes of serum to one volume of washed packed cells) for 30 minutes at 4°C. The absorbed serums were then tested on embryos at a dilution of 1 : 8 (Table 1). The reactivity of the antiserum to H-Y was completely lost after absorption with male cells, but the cytotoxic potential was only slightly reduced after absorption with female cells. These findings demonstrate the specificity of the antiserum for the H-Y antigen.

In addition, four-cell (1-day-old) and 10- to 16-cell (2.5- to 3-day-old) embryos were examined for the presence of H-Y. Fifty percent of the 10- to 16-cell embryos were susceptible to the cytolytic action of the H-Y antibody; however, H-Y antigen was not detectable on the four-cell embryo.

Our results support the findings of Wudl and Chapman (*6*) showing that paternal genes are expressed prior to implantation during the development of mouse embryos. Furthermore, this work provides additional evidence that the expression of H-Y antigen is dependent on the presence of the Y chromosome as suggested by Bennett *et al.* (*3*) and confirms their results that the expression of H-Y on male cells is not dependent on testosterone.

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Coronary Arterial Smooth Muscle Contraction by a Substance Released from Platelets: Evidence That It Is Thromboxane A_2

Abstract. *When human platelets are aggregated by thrombin, material is released that rapidly contracts strips of spirally cut porcine coronary artery. Prevention of the contraction by indomethacin suggested mediation by a prostaglandin. The contraction produced by aggregating platelets was unlike those produced by prostaglandins E_2, $F_{2\alpha}$, G_2, or H_2, but resembled that evoked by thromboxane A_2, which is formed by platelets during aggregation.*

Evidence suggests that some cases of transient myocardial ischemia at rest result from constriction of the larger coronary arteries (*1*). An acute reduction in flow resulting from coronary constriction could also conceivably set the stage for myocardial infarction or fatal cardiac arrhythmias.

The intimate involvement of platelets in the development of atherosclerotic lesions (*2*) led us to investigate the possibility that platelet aggregation could interrupt coronary flow by releasing a substance that constricts the major coronary arteries.

To investigate the possible release of vasoconstrictors from platelets, we placed suspensions of washed human platelets that had been stimulated to aggregate into baths containing isolated coronary arteries. Washed platelets were prepared from blood of normal male volunteers who had not taken aspirin for at least 1 week (*3*). The left coronary artery was removed from fresh porcine hearts, spirally cut into strips and placed in Krebs-Ringer bicarbonate buffer (37°C) saturated with 95 percent O_2 and 5 percent CO_2 and containing 8 μM indomethacin. After 3 hours of equilibration, changes in isometric tension were measured in arterial strips that were under tension (1.25 to 1.50 g). Contractile responses were expressed relative to the maximum tension developed with 85 nM prostaglandin E_2 (PGE_2). Washed platelets were incubated in a Payton aggregation module and then stimulated to aggregate with thrombin (1 unit per milliliter of washed platelets). At various times after the addition of thrombin, 0.75 ml of the platelet suspension was removed from the aggregation module, quickly diluted to 3 ml with bathing medium and transferred to 3-ml coronary artery baths. In some experiments, the synthesis of prostaglandins was inhibited by adding indomethacin (27 μM) (Fig. 1) to the platelets before thrombin stimulation. Because aggregating platelets also release 5-hydroxytryptamine (5-HT), the artery strips were first treated with the 5-HT antagonist, dihydroergotamine (0.7 μM), except where indicated otherwise.

After the addition of thrombin to the platelets, maximum amounts of a contractile substance existed within 15 seconds (Fig. 2). The contraction onset was rapid and reached a maximum in 1.1 ± 0.1 minutes (mean ± standard error; N = 8) as shown in Fig. 3A. Two minutes after thrombin was added to the platelets, the evoked coronary contraction was smaller, and by 4 to 8 minutes no contraction was induced. These data suggested that a labile contracting substance was released by the platelets in the presence of thrombin. Indomethacin, which inhibits prostaglandin formation but not prostaglandin action, prevented contraction, suggesting that the contractile substance was a prostaglandin. The contractions produced by 255 nM PGE_2 (Fig. 3B) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were, in contrast to those produced by thrombin-stimulated platelets, slow to develop and persisted long after 4 minutes. Prior treatment of the strips with dihydroergotamine unmasked an unidentified relaxing factor which lowered the baseline tension but did not block the contraction produced by thrombin-induced aggregation.

The prostaglandin cyclic endoperoxides G_2 and H_2 (PGG_2, PGH_2, Fig. 1) contract isolated rabbit aortic smooth muscle (*3, 4*). To test the effects of these cyclic endoperoxides on coronary smooth muscle, PGG_2 and PGH_2 were synthesized from arachidonic acid by using the cyclooxygenase from sheep seminal vesicular microsomes (*3*). After they were extracted from the incubation mixture, PGG_2 and PGH_2 were separated by silicic acid chromatography (*3*). Twenty

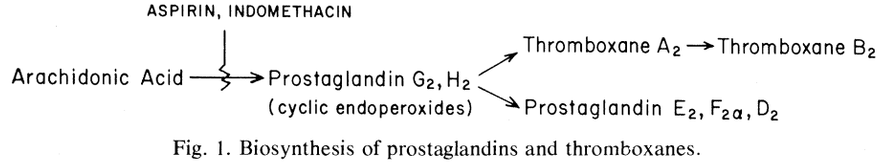

Fig. 1. Biosynthesis of prostaglandins and thromboxanes.

Table 1. Rate of TxB_2 formation by washed platelets stimulated to aggregate by addition of thrombin. Results are expressed as nanomoles of TxB_2 per milliliter of washed platelets per minute.

Time after addition of thrombin (minutes)	Rate of TxB_2 formation (nmole ml^{-1} min^{-1})
0 to 0.25	2.51
0.25 to 0.5	1.29
0.5 to 1.0	0.61
1.0 to 2.0	0.48
2.0 to 3.0	0.22
3.0 to 4.0	0.15

percent of the incubated arachidonic acid was recovered as cyclic endoperoxides. The structures of PGG_2 and PGH_2 isolated from this biosynthetic procedure were confirmed by mass spectrometry of their respective reduction and isomerization products (*3*). Thin-layer chromatography of the ^{14}C-labeled $PGF_{2\alpha}$ formed by reduction of PGG_2 and PGH_2 indicated that the endoperoxides were at least 90 percent pure. Both PGG_2 and PGH_2 (255 n*M*, Fig. 3C) produced an initial small contraction followed by a slowly increasing contraction similar to that produced by 255 n*M* PGE_2 (Fig. 3B). At the peak of contraction produced by PGH_2, the bathing medium was drained and a deuterated PGE_2 internal standard was added to the medium. The medium was then extracted with chloroform (*p*H 3) and PGE_2 was isolated by high-pressure liquid chromatography. Subsequent quantification of PGE_2 by combined gas chromatography and mass spectrometry revealed that 93 percent of the PGH_2 which had been added to the bath had been converted to PGE_2.

Thromboxane A_2 (TxA_2) is one of the principle biologically active prostaglandins released from aggregating platelets and has a half-life of 32 seconds in an aqueous medium (*5*) (Fig. 1). When generated by the addition of cyclic endoperoxide to indomethacin-treated platelets, TxA_2 induces contraction of aortic strips of the rabbit (*5*). To determine whether the fast coronary contraction produced by thrombin-stimulated platelets was characteristic of TxA_2, we used the particulate fraction from platelets to convert cyclic endoperoxides into TxA_2. Washed, human platelets were frozen and thawed three times, homogenized, and centrifuged at 2000*g* for 15 minutes. The 2000*g* supernatant fluid was then spun at 100,000*g* for 1 hour, and the resulting pellet was resuspended in a volume of Krebs-Ringer bicarbonate buffer equivalent to one-fifth of the washed platelet volume. Portions (0.05 ml) of the suspension of platelet particles were added to the coronary artery baths containing 2.95 ml of medium, then PGG_2 or PGH_2 was added until the final concentration was 255 n*M*.

The addition of PGG_2 to the particulate fraction (N = 21) resulted in a contraction that reached a maximum in 1.1 ± 0.1 minutes (mean ± standard error) with a rate of tension development that was 4.7 times that produced when PGG_2 alone was added to the medium. The contraction produced by adding PGG_2 to the medium reached maximum tension in 9.2 ± 0.5 minutes. Similarly, the contraction induced by the addition of PGH_2 to the particulate fraction (N = 23) attained a maximum in 1.3 ± 0.1 minutes (Fig. 3D) and had a rate of tension generation that was 3.3 times that of PGH_2 alone. The contraction when PGH_2 was added to the medium reached its maximum tension in 8.5 ± 0.4 minutes. When PGH_2 or PGG_2 was incubated with the platelet particulate fraction for 1 minute before being added to the coronary arteries, no contraction occurred, indicating that the contractile substance was labile, similar to TxA_2. The addition of thromboxane B_2 (TxB_2), the metabolite of TxA_2, in concentrations up to 3000 n*M* did not induce coronary artery contraction. The substance in unstimulated or indomethacin-blocked platelets that caused relaxation of dihydroergotamine-treated strips was localized in the 100,000*g* supernatant fluid from disrupted platelets. When PGG_2 or PGH_2 was added to baths already containing the 100,000*g* supernatant fluid, the relaxed strips did not contract or contracted less than when PGG_2 or PGH_2 was added to coronary artery baths containing only normal bathing medium. The addition of only platelet particles to the baths did not alter coronary artery tension.

To confirm that the platelet particulate fraction formed TxA_2 from PGH_2, we

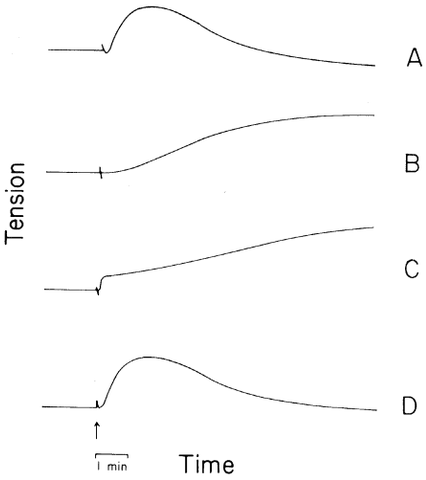

Fig. 3. Representative tracings showing the pattern of tension changes in 5-HT blocked (0.7 μ*M* dihydroergotamine) coronary arteries in response to the addition of: (A) washed platelets stimulated with thrombin for 30 seconds; (B) 255 n*M* PGE_2; (C) 255 n*M* PGH_2; and (D) platelet particles plus 255 n*M* PGH_2. All tracings have the same tension scale.

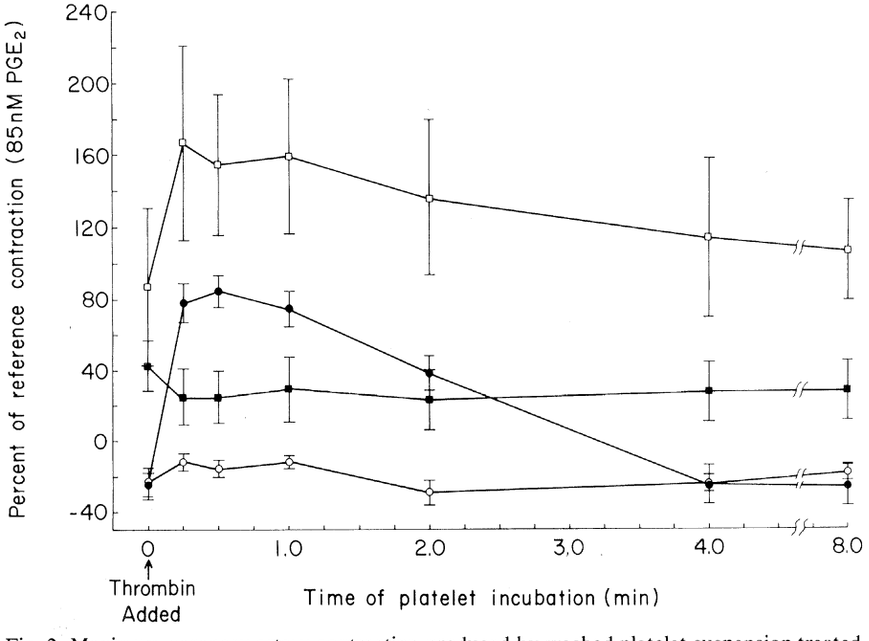

Fig. 2. Maximum coronary artery contraction produced by washed platelet suspension treated with thrombin for varying periods of time. Each point represents the mean of eight different artery strips ± standard error. No blocking agent, □; platelet PG synthesis blocked with 27.0 μ*M* indomethacin, ■; coronary response to 5-HT blocked with 0.7 μ*M* dihydroergotamine and platelet PG synthesis blocked with 27.0 μ*M* indomethacin, ○; coronary response to 5-HT blocked with 0.7 μ*M* dihydroergotamine, ●.

identified TxB_2 in the bathing medium. After extraction and isolation of TxB_2 by high-pressure liquid chromatography, we obtained by combined gas chromatography and mass spectrometry the expected mass spectrum of the methyl ester of the methoxime trimethylsilyl ether derivative (*6*). The quantity of TxB_2 formed in the bath was determined by using octadeutero-TxB_2 as a carrier and selectively monitoring the ratios of *m/e* of the ions 301 and 304 (*7*). Three minutes after PGH_2 (255 n*M*) was added to the muscle bath containing 2.95 ml of buffer and 0.05 ml of the platelet particulate suspension, the muscle bath was found to contain 52 n*M* TxB_2.

Having shown that the TxA_2 generating system produced a contraction similar to that evoked by aggregating platelets, we assessed the formation of TxA_2 by whole platelets aggregated under these conditions by measuring TxB_2 in the aggregation module at various times after the addition of thrombin. If one assumes that the reaction follows first order kinetics, the rate of TxB_2 formation should parallel the concentration of TxA_2. The rate of TxB_2 formation reached a maximum in less than 15 seconds after thrombin addition to the platelets and essentially ceased after 3 to 4 minutes (Table 1). Thus the time course of the contractions produced by aggregating platelets are a reflection of the concentration of TxA_2, indicating concomitant variation in the levels of the putative mediator and the response.

We conclude that TxA_2 released from aggregating platelets can contract coronary smooth muscle in vitro. These findings provide the basis for the following hypothesis: Platelet aggregation in areas of damaged endothelium can release TxA_2 and thus cause constriction of large coronary arteries. This hypothesis merits testing in conditions where coronary spasm is known or postulated (initiation of myocardial infarction, sudden cardiac death, and variant angina).

More generally, these findings suggest that TxA_2 may be a factor in other forms of arterial constriction in which platelets participate. The cerebral arterial spasm associated with subarachnoid hemorrhage is of particular interest, as we have found that the TxA_2-generating system produces contraction of bovine intracranial arterial strips (*8*).

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Microbial Degradation of Condensed Tannins

Abstract. *A strain of* Penicillium adametzi *Zaleski was isolated from enrichment cultures with condensed tannins as the carbon source. Low-molecular-weight condensed tannins, extracted and purified from* Pinus radiata *bark, were used as substrates for quantitative growth measurements on this fungus in defined culture conditions.*

The well-known ability of plant tannins to inhibit the growth of microorganisms has been attributed to the capacity of these substances to bind strongly to proteins and polysaccharides. Enzymes are wholly or partially inactivated by complex formation with tannins (*1*), while potential microbial substrates such as polysaccharides and nonenzyme proteins become highly resistant to microbial attack after binding to tannin molecules (*2, 3*). The significance of these effects of tannins in relation to plant disease resistance and to the rates of decomposition of organic molecules in soil was reviewed by Starkey and his colleagues (*2, 3*), who also found that several of the condensed tannins were themselves very resistant to microbial attack. It is nevertheless evident that microorganisms capable of degrading such a ubiquitous natural product must exist, although, considering the obvious importance of such organisms in soil biochemistry and microbial ecology, it is perhaps surprising that very little work has been published in this area (*4*). I describe here for the first time the isolation of an organism capable of growth on condensed tannins as sole carbon source under defined culture conditions. This work forms part of a study on the microbial breakdown of *Pinus radiata* bark, a major waste product of the New Zealand forestry industry. Condensed tannins and related phenolic polymers account for 50 to 60 percent of the dry weight of this bark (*5, 6*).

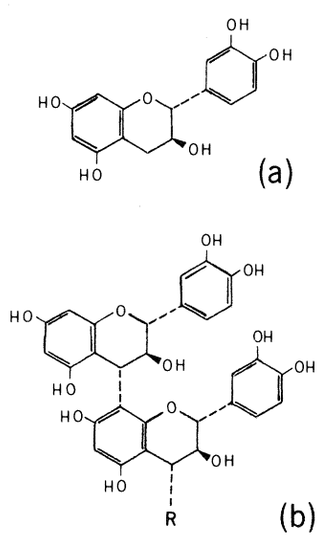

Fig. 1. Structures of (a) (+)-catechin and (b) the procyanidin dimer, B-3 (R = H), and the trimer, C-2 [R = (+)-catechin].

Enrichment culture experiments were conducted with a purified preparation of condensed tannins (*7*) as sole carbon source in a mineral salts medium (*8*). Temperature, *p*H, tannin concentration, nitrogen source, and time of incubation were varied in repeated attempts to isolate tannin-degrading organisms from several forest and garden soil samples, and from samples of rotting wood and bark. Ultimately an inoculum from a rotting *Pinus radiata* log yielded a filamentous fungus apparently capable of growth on condensed tannins as its sole carbon source. This fungus has since been classified by the Commonwealth Mycological Institute, Kew, London, as *Penicillium adametzi* Zaleski, and has been given the catalog number IMI 198150.

Although it is now generally accepted that condensed tannins are polymers of flavan-3-ols such as (+)-catechin (Fig. 1a) or flavan-3,4-diols such as leucocyanidin (*9*), no individual high-molecular-weight condensed tannin polymer has ever been isolated in the pure state. Previous microbiological studies with these substances (*2–4*) have used partially purified plant extracts, that is, tannin mix-