# Localization of Platelet Prostaglandin Production in the Platelet Dense Tubular System

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**Platelet production of 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and 8-(1-hydroxy-3-oxopropyl)-9, 12L-dihydroxy-5,10-heptadecadienoic acid (PHD), two metabolites of the prostaglandin cyclic endoperoxides $PGG_2$ and $PGH_2$, was found in this investigation to occur primarily in a platelet microsomal fraction consisting almost exclusively of membranes. To further localize the membrane site of platelet prostaglandin biosynthesis, the present study has used a cytochemical technique employing 3,3'-diaminobenzidine as an oxidizable substrate. The reaction product was found to localize in the platelet dense tubular system. Formation of the reaction product was inhibited by aminotriazole. In similar concentrations, aminotriazole inhibited collagen and arachidonic acid aggregation, the second wave of ADP and epinephrine aggregation, but failed to inhibit aggregation by $PGG_2$ and A23187. A study of the mechanism of action of aminotriazole revealed inhibition of formation of HHT and PHD. The results localize platelet prostaglandin biosynthesis to the membranes of the dense tubular system. (Am J Pathol 83:283–298, 1976)**

A PRODUCT OF PLATELET prostaglandin biosynthesis plays a major role in platelet activation.[1-3] Studies have shown that arachidonic acid is converted by platelets into two prostaglandin endoperoxides, $PGG_2$ and $PGH_2$.[4] The endoperoxides are subsequently metabolized to 8-(1-hydroxy-3-oxopropyl)-9, 12 L-dihydroxy-5,10-heptadecadienoic acid (PHD) and 12 L-hydroxy-5,8,10-heptadecatrieonoic acid (HHT). Recent studies have shown that an active compound, most likely thromboxane $A_2$, which is an intermediate between the endoperoxides and PHD formed on addition of arachidonic acid to platelet microsomes, serves as an intracellular and intercellular messenger promoting platelet stickiness and triggering the platelet release reaction.[5-9] Results of these investigations have suggested that thromboxane $A_2$ may act as a physiologic ionophore to mobilize and transport calcium to sites of contractile activity.[10] Since the origin of a chemical mediator within a cell may be critical to its action, it is important to localize its site of synthesis.

Methods used to localize the site of prostaglandin biosynthesis in other

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tissues have included subcellular fractionation and histochemistry. In the sheep vesicular glands, prostaglandin biosynthesis occurs primarily in the columnar epithelial cells lining the lumina of the secretory units,[11] and has been localized to a microsomal fraction within cells of the sheep vesicular gland.[12] In the rabbit renal papilla, prostaglandin $E_2$ was found to be formed in membranes which sediment in the microsomal fraction and which might be derived from either the cell membranes or membranes of the endoplasmic reticulum.[13] Similar findings have been made in skin, and in the rat cerebral cortex.[14,15] In this paper, we report on the transformation of arachidonic acid by fractionated platelets, followed by cytochemical studies to probe the intracellular site of platelet prostaglandin synthesis.

## Materials and Methods

### General

The procedures used in this laboratory to obtain blood from normal donors and mix the samples immediately with 3.8% trisodium citrate or citrate-citric acid, pH 6.5, in a ratio of nine parts blood to one part anticoagulant, separate platelet-rich plasma (C-PRP) by centrifugation at 100*g* for 20 minutes at room temperature, expose samples of normal platelet-rich plasma on the platelet aggregometer to various drugs and experimental conditions, and prepare samples for transmission electron microscopy by fixation in 0.1% glutaraldehyde and 3% glutaraldehyde in White's saline, then in 1% osmic acid before dehydration and embeddment in Epon, have been described in recent publications.[16-18] Thin sections were cut and examined prior to and after staining with lead citrate and uranyl acetate in a Philips 301 electron microscope.

### Cytochemical Localization of Peroxidase in Normal Platelets

Peroxidase was localized in platelets employing a modification of the method of Graham and Karnovsky.[19] Samples of C-PRP were initially fixed at 37 C with 0.1% glutaraldehyde in 0.1 M cacodylate buffer containing 5% sucrose, then in 2.5% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer without sucrose at room temperature. The fixed samples were washed three times with Tris-HCl buffer at pH 6.0 and then incubated for 60 minutes at room temperature in 0.05 M Tris buffer containing 2.5 mg/ml diaminobenzidine, 0.03% hydrogen peroxide, and 1% dimethylsulfoxide. Reacted platelets were washed twice in Tris-HCl buffer pH 6.0 and fixed for 1 ½ hours in 2% osmic acid in 0.1 M cacodylate buffer, before dehydration and embedding.[20] To enhance visualization of the peroxidase reaction product, sections studied for peroxidase activity were not stained with lead citrate and uranyl acetate.

### Preparation of Platelet Fractions

Platelet fractions were prepared after twice washing, freeze-thawing, and sonicating the platelets as described earlier.[8] The resultant platelet homogenate in 0.1 M Tris buffer, pH 8.0, was centrifuged at 900*g* for 20 minutes to obtain a fraction enriched in dense bodies (Figure 1A). The supernatant was centrifuged at 13,000*g* for 20 minutes to obtain a fraction enriched in granules (Figure 1B), and the supernatant from this spin was sedimented at 105,000*g* for 1 hour to obtain a membrane or microsomal fraction (Figure 1C). Each platelet fraction was washed once and then resuspended in 0.1 M Tris buffer, pH 8.0,

so that 1 ml of each fraction was derived from an equal amount of the initial platelet homogenate (an amount derived from $4 \times 10^8$ platelets).

### Measurement of Platelet Production of HETE, HHT, and PHD

Production of 12L-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), HHT, and PHD was assessed using the method of Hamberg and Samuelsson.[21] $^{14}$C-arachidonic acid (greater than 99% pure, Applied Science) was diluted with cold arachidonic acid (greater than 99% pure, Nucheck Prep) to a specific activity of 25 to 28 mCi/mM, made up as a sodium salt as described previously,[8] and diluted in 0.1 M Tris buffer, pH 8.0. One-milliliter aliquots of each platelet fraction were then incubated for 5 minutes at 37 C with identical amounts of $^{14}$C-arachidonic acid (about 500 ng). At the end of each incubation period, 10 ml of ethanol was added to stop the reaction. The samples were then acidified with 1 N HCl to pH 3.0, diluted with water, and extracted into diethyl ether. Magnesium sulfate ($MgSO_4$) was added to the ether to remove water. The metabolites were methylated using diazomethane and were run on a thin layer plate using the organic layer of isooctane, ethyl acetate, and water (100:50:100). The thin layer plates were run on a thin layer radiochromatogram scanner and the production of the metabolites was assessed by scraping counting the radioactivity in each fraction. Since the HETE and HHT fractions could not be easily separated by scraping alone, the two were scraped together and the total counts in this fraction divided according to the relative peak heights from the thin layer radiochromatogram. Four aliquots of $^{14}$C-arachidonic acid incubated with buffer alone and then extracted and plated in the same fashion were used to give a figure for the background radioactivity (less than 1% of total) in the same region which was subtracted from the counts in each fraction. Statistical comparison of the production of metabolites by the various fractions was done using the Student *t* test. Mass spectrometry was used to confirm the presence of HETE, HHT, and PHD in the peaks of the thin layer plate.[7] Control samples containing the methylated derivatives of HETE, HHT, and PHD; HETE alone; and arachidonic acid alone were run with each experiment to monitor the position of the peaks on the thin layer radiochromatogram.

### Experiments With Aminotriazole

Aminotriazole (Schwartz-Mann Chemical Co.) was made up as a 1 M solution in distilled water and diluted in Hanks' balanced salt solution (HBSS) to the required strength. Experiments using aminotriazole as an inhibitor were done following incubation of C-PRP or washed platelets with the aminotriazole for 30 minutes. For assessment of the effects of aminotriazole on arachidonic acid–induced aggregation and prostaglandin synthesis, platelets were washed three time, pelleted at 900*g* and 4 C in the presence of 1% EDTA, and resuspending in Hanks' balanced salt solution, pH 7.4, without calcium and magnesium. For the aggregation studies, 0.5 mM calcium was added to the washed platelets, and the pH of the platelet suspension, aminotriazole solution, and arachidonic acid to be added were adjusted to pH 8.0 (optimum pH for arachidonic acid–induced aggregation).[7] Collagen, aspirin, and indomethacin were prepared as described earlier.[18,22] Prostaglandin $G_2$ was prepared using a modification of the method of Hamberg *et al.*[4] The sheep vesicular gland microsome and arachidonic acid incubation was terminated by pouring the reaction mixture into a separatory funnel containing 60 ml of isopropyl alcohol and 100 ml of diethyl ether. Then 300 ml of 25 mM HCl was added. After extraction, the upper phase was evaporated to dryness, and the residue was redissolved and subjected to silicic acid chromatography according to Hamberg *et al.*[4] The prostaglandin $G_2$ was characterized by its elution profile on silicic acid chromatography, its lability in aqueous solution, the ability of low concentrations to aggregate aspirin- and indomethacin-treated platelets and by its reduction with stannous chloride, $SnCl_2$, to radioimmunoassayable prostaglandin $F_{2\alpha}$.

All experiments reported in this paper were performed on more than one occasion with similar results. Since the peroxidase reaction product can occasionally give false negative results, the inhibition of the cytochemical demonstration of peroxidase by aminotriazole was repeated five times with the same results.

## Results

### Transformation of Arachidonic Acid by Subcellular Platelet Fractions

Analysis of the production of the platelet metabolites of arachidonic acid in the several platelet fractions revealed that production of HHT and PHD, metabolites of the prostaglandin endoperoxides $PGG_2$ and $PGH_2$, was the highest in the microsomal fraction containing platelet membranes (Table 1). Significantly less of these metabolites ($P < 0.01$) was found in any of the other fractions. In contrast, production of HETE was highest in the 105,000*g* supernatant ($P < 0.001$). The finding that arachidonic lipoxygenase (the enzyme responsible for conversion of arachidonate to HETE) is a soluble one has also been made recently by Nugteren.[23]

### Cytochemical Localization of the Site of Platelet Prostaglandin Biosynthesis

#### Platelet Peroxidase and Its Inhibition by Aminotriazole

Panganamala and co-workers suggested that a peroxidase may be needed for prostaglandin biosynthesis.[24] This led us to evaluate platelet peroxidase using a modification of the method of Graham and Karnovsky.[19] Platelets prepared in this fashion showed selective staining of the

Table 1—Arachidonic Acid and Metabolites Produced Following Addition of $^{14}$C-Arachidonic Acid to Platelet Fractions

| | Fraction | | | |
|---|---|---|---|---|
| | I Dense body-enriched fraction (900*g* pellet) | II Granule enriched fraction (13,000*g* pellet) | III Microsomal fraction (105,000*g* pellet) | IV Soluble fraction (105,000*g* supernatant) |
| AA | 55.5 ± 0.72 | 46.3 ± 0.69 | 22.3 ± 1.73 | 39.1 ± 1.38 |
| HETE | 17.8 ± 0.76 | 12.6 ± 1.17 | 7.4 ± 0.38 | 47.4 ± 1.00 |
| HHT | 13.3 ± 0.57 | 23.8 ± 2.11 | 46.6 ± 1.55 | 6.7 ± 0.69 |
| PHD | 13.5 ± 0.46 | 17.3 ± 0.99 | 23.6 ± 1.13 | 6.8 ± 0.29 |

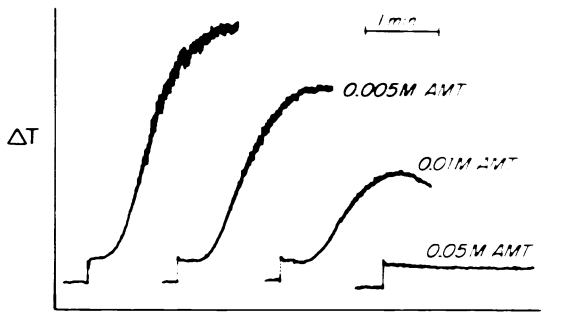
Proportion of radioactivity as AA, HETE, HHT or PHD is expressed as mean ± SE of four replicates.

The transformation of arachidonic acid by platelet fractions. Conversion of arachidonic acid to HHT and PHD was greater in the microsomes than in any other fraction ($P < 0.01$). Formation of HETE from arachidonic acid was greater in the soluble fraction than in any other fraction ($P < 0.001$).

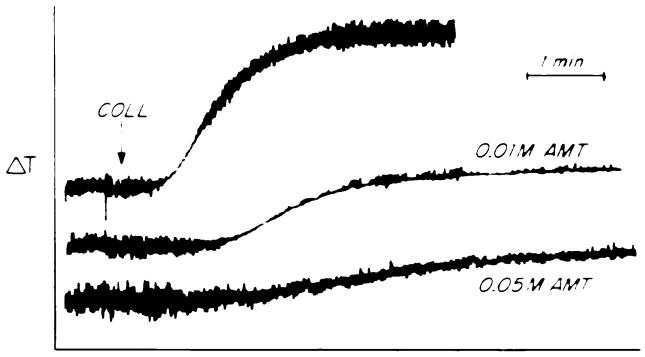
membranes of the dense tubular system (Figure 2). No reaction product was seen in platelets incubated in media without DAB, in media without hydrogen peroxide, or with a marked excess of $H_2O_2$. The reaction product could be found in the dense tubular system if the platelets were incubated with DAB and $H_2O_2$ prior to fixation, though preservation of platelets ultrastructure was less than optimal. The reaction was better at pH 6.0 than at pH 9.0; 0.05 M aminotriazole incubated with the platelets for 30 minutes prior to fixation completely inhibited the deposition of stain in the dense tubular system (Figure 6). With 0.02 M aminotriazole, inhibition was partial (Figure 5); with 0.01 M aminotriazole, considerable peroxidase activity was still apparent (Figures 3 and 4). Deposition of the reaction product in the dense tubular system was not inhibited by 100 $\mu$g/ml aspirin or 100 $\mu$g/ml indomethacin.

### The Influence of Aminotriazole on Platelet Aggregation

Inhibition of platelet aggregation by aminotriazole was concentration dependent. At concentrations of 0.05 M to 0.1 M, aminotriazole completely inhibited arachidonic acid–induced platelet aggregation and strongly inhibited collagen aggregation (Text-figures 1 and 2); 0.05 M aminotriazole concentrations inhibited the second wave of epinephrine and ADP induced aggregation, but did not inhibit aggregation induced by 250 ng prostaglandin $G_2$ or 20 $\mu$M A23187. Lower concentrations of aminotriazole (0.01 M and 0.005 M) produced partial inhibition of arachidonic acid aggregation, weak inhibition of collagen aggregation, and variable inhibition of the second wave of epinephrine and ADP aggregation. Inhibition of aggregation was found to coincide with inhibition of the cytochemical reaction, resulting in staining of the platelet dense tubular system for the reaction product of peroxidase activity.




TEXT-FIGURE 1—Evaluation of the influence of aminotriazole (AMT) on arachidonic acid–induced aggregation of washed platelets. At a concentration of 1.7 $\mu$M, arachidonic acid produced a rapid and irreversible single wave of aggregation. Incubation of the platelets with 0.005 M or 0.01 M aminotriazole for 30 minutes caused partial inhibition of arachidonic acid aggregation. Incubation of the platelets with 0.05 M aminotriazole completely inhibited arachidonic acid aggregation. $\Delta$ T = change in light transmission.




TEXT-FIGURE 2—The influence of aminotriazole *AMT* on collagen *COLL* -induced aggregation. Addition of collagen to platelet-rich plasma produced a single wave of irreversible aggregation. Incubation of the platelet-rich plasma with 0.01 M aminotriazole for 30 minutes partially inhibited aggregation by collagen. Marked inhibition of collagen aggregation occurred at a concentration of 0.05 M aminotriazole. ΔT = change in light transmission.

In conjunction with an assessment of the influence of aminotriazole on platelet aggregation, we assessed the effect of this agent on platelet ultrastructure. Platelets stirred with collagen in the presence of 0.01 M aminotriazole showed large aggregates (Figures 3 and 4). Platelets within these aggregates showed shape change, internal transformation with centralization of granules, and some degranulation. Staining of these platelets revealed considerable peroxidase activity in the dense tubular system. Platelets stirred with collagen in the presence of 0.02 M aminotriazole showed smaller aggregates and many single platelets. Almost all platelets showed shape change, but few showed the centripetal movement of granules closely surrounded by the microtubule bundle, essential features of the contractile wave (Figure 5). Platelets stirred with collagen in the presence of 0.05 M aminotriazole retained their resting discoid shape, did not aggregate, and did not reveal reaction product in the dense tubular system (Figure 6).

### The Influence of Aminotriazole on the Transformation of Arachidonic Acid by Platelets

The pattern of inhibition of platelet aggregation by aminotriazole was similar to that seen by prostaglandin synthetase inhibitors. Therefore, prostaglandin synthesis in platelets exposed to various concentrations of aminotriazole was determined by quantitating the transformation of arachidonic acid to HHT and PHD. Aminotriazole inhibited formation of HHT and PHD by platelets in a concentration-dependent fashion but did not inhibit production of HETE. At concentrations of 0.05 M and 0.1 M aminotriazole, platelet production of PHD and HHT was almost completely inhibited (Table 2, Text-figure 3). At concentrations of 0.01 M and

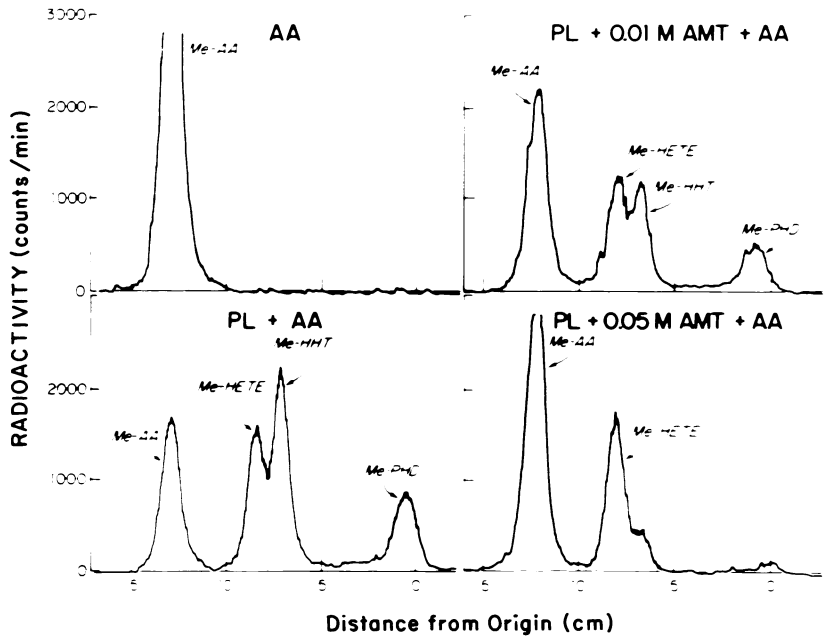
Table 2—The Influence of Aminotriazole on Platelet Production of HETE, HHT, and PHD

| | Concentration of aminotriazole | | | | |
|---|---|---|---|---|---|
| | 0 | 0.005 M | 0.01 M | 0.05 M | 0.1 M |
| AA | 23.3 ± 0.6 | 38.7 ± 2.9 | 43.7 ± 1.1 | 68.5 ± 5.0 | 62.1 ± 1.5 |
| HETE | 19.4 ± 1.7 | 24.0 ± 1.6 | 24.1 ± 1.3 | 24.9 ± 3.8 | 32.2 ± 1.5 |
| HHE | 39.4 ± 3.6 | 25.4 ± 1.3 | 22.6 ± 0.2 | 5.4 ± 1.3 | 4.2 ± 0.6 |
| PHD | 17.9 ± 1.1 | 11.8 ± 1.2 | 9.6 ± 0.9 | 1.2 ± 0.5 | 1.8 ± 0.4 |

Proportion of radioactivity as AA, HETE, HHT, or PHD is expressed as mean ± SE of four replicates.

The effect of aminotriazole on the transformation of arachidonic acid by platelets. Aminotriazole at a final concentration of 0.005 M or 0.01 M produced partial inhibition of formation of HHT and PHD from $^{14}$C-arachidonic acid, while 0.1 M and 0.05 M aminotriazole produced more than 85% inhibition of the conversion of arachidonic acid to HHT and PHD.

0.005 M, aminotriazole partially inhibited production of PHD and HHT. The results show that aminotriazole inhibits synthesis of $PGG_2$ and $PGH_2$ by platelets in similar concentrations to its effect on platelet aggregation and the cytochemical demonstration of platelet peroxidase.




TEXT-FIGURE 3—The influence of aminotriazole (AMT) on the transformation of arachidonic acid (AA) by platelets. A thin layer radiochromatogram of $^{14}$C-arachidonic acid stirred for 3 minutes with HBSS on an aggregometer (upper left) reveals a single peak. Stirring the $^{14}$C-arachidonic acid (approximately 1.7 μM) with washed platelets on an aggregometer (lower left) results in the formation of HETE, HHT, and PHD. Incubation of the platelets with 0.01 M aminotriazole prior to addition of the arachidonic acid partially inhibited transformation to HHT and PHD (upper right). Aminotriazole at a final concentration of 0.05 DM almost completely inhibited conversion of arachidonic acid to HHT and PHD (lower right).

## Discussion

The present study has investigated the site of prostaglandin biosynthesis in platelets. An initial step in this investigation was to fractionate platelets and study the transformation of arachidonic acid by platelet fractions. Production of HHT and PHD was highest in a microsomal fraction containing membranes. The finding is not an artifact related to decay of the enzyme since the microsomal fraction of necessity took longest to prepare, and as a result was processed last. Our results eliminated dense bodies, granules, and cytosolic enzymes as major sites of platelet prostaglandin biosynthesis. Similar observations have been made in studies of sheep vesicular glands,[12] rabbit renal papilla,[13] human skin,[14] and rat cerebral cortex,[15] where prostaglandin biosynthesis has been shown to occur in the microsomal fraction. Earlier work on platelets in this laboratory [9,25] utilized a microsomal fraction to synthesize the active platelet prostaglandin from arachidonic acid which suggested that platelet prostaglandin synthesis occurs in the microsomes. However, no comparative studies of the various fractions were done in the earlier investigations. Since the platelet microsomal fraction used in our experiments included a mixture of plasma membranes, organelle membranes, membranes of the open canalicular system, and membranes of the dense tubular system, it was essential to distinguish among them in order to further localize platelet prostaglandin biosynthesis.

The next step in our efforts to localize the intracellular site of prostaglandin synthesis involved an investigation of platelet peroxidase, an enzyme which has been tentatively implicated in prostaglandin biosynthesis [24] and which has been localized in previous investigations to the dense tubular system.[20] Cavallo studied this enzyme in rabbit renal medulla and suggested that it might be linked to prostaglandin biosynthesis.[26] Janszen and Nugteren used a histochemical procedure employing diaminobenzidene to localize prostaglandin biosynthesis in the columnar epithelial cells of the sheep vesicular gland and in cells of the renal medulla.[11,27] It seemed likely, therefore, that a modification of Graham and Karnovsky's technique to localize peroxidase might be very useful in the present investigation.

Earlier studies with this technique showed that the reaction product was localized to the membranes of the dense tubular system.[20] Since studies with platelets suggested that platelet prostaglandin synthesis occurred within the cell rather than at the external membranes,[28] this seemed a promising area to investigate. If the platelet enzyme involved in the oxidation of diaminobenzidine was important to the physiologic ag-

gregation of platelets, we reasoned that an agent which inhibited this reaction (aminotriazole) should inhibit platelet function. Aminotriazole was found to inhibit collagen aggregation in concentrations similar to those which inhibited cytochemical demonstration of peroxidase activity suggesting that the two were closely linked. A study of the influence of aminotriazole on other aggregating agents revealed a pattern of inhibition similar to that produced by other agents which inhibit prostaglandin synthesis. Furthermore, ultrastructural studies of inhibition by aminotriazole revealed that the agent blocked the ultrastructural changes induced by collagen in a fashion similar to aspirin and indomethacin,[22] agents known to inhibit prostaglandin biosynthesis.[21,29]

These studies led to direct analysis of the effect of aminotriazole on prostaglandin biosynthesis. We found that aminotriazole inhibited prostaglandin biosynthesis in concentrations similar to those which blocked platelet aggregation. When coupled with the evidence that aggregation by the prostaglandin endoperoxide $PGG_2$ was not inhibited by aminotriazole, the result shows that aminotriazole inhibits platelet aggregation by specifically inhibiting the conversion of arachidonic acid to prostaglandin $G_2$. The inhibition by aminotriazole of the deposition of the diaminobenzidine reaction product in the dense tubular system in similar concentrations to its inhibition of platelet aggregation and prostaglandin biosynthesis links these three processes, and suggests that the oxidative enzyme present in the dense tubular system is involved in platelet prostaglandin biosynthesis.

The role of the dense tubular system oxidative enzyme in prostaglandin synthesis is not yet clear. Since neither aspirin nor indomethacin inhibited the oxidation of diaminobenzidine and the formation of reaction product, this enzyme is not the cyclooxygenase per se but rather a closely linked enzyme. This conclusion is supported by evidence that the pH optimum of the platelet peroxidase, pH 6.0–7.5, is not the same as the pH optimum for the cyclooxygenase, pH 8.0.[7] The enzyme involved is not catalase since the staining conditions for catalase are different,[30] and since aminotriazole does not inhibit platelet catalase.[31] The present investigation is consistent with the view that the enzyme is a peroxidase and our findings fit with those of Panganamala *et al.* who initially suggested that a peroxidase is involved in synthesis of prostaglandins by platelets.[24,32] More recent findings of Vargaftig, Tranier, and Chignard support this conclusion.[33] Further investigations of this enzyme and its role in platelet prostaglandin biosynthesis are in progress.

The finding that platelet prostaglandin production occurs in the dense tubular system fits well with the hypothesis that a short-lived product of

platelet prostaglandin biosynthesis, perhaps thromboxane $A_2$, is a physiologic ionophore.[10] The dense tubular system, a smooth endoplasmic reticulum membrane system, is the platelet sarcoplasmic reticulum and site of calcium sequestration.[20] In muscle cells, contraction is triggered by the movement of calcium from the sarcoplasmic reticulum to the contractile proteins.[34] Evidence developed in this laboratory suggests that platelet contraction is most likely triggered by the active product of prostaglandin synthesis which transports calcium from the dense tubular system where prostaglandins are synthesized into the cytoplasm where the contractile proteins are located. Understanding the site of synthesis of prostaglandins in platelets has, therefore, important implications for the action of prostaglandins in platelets.

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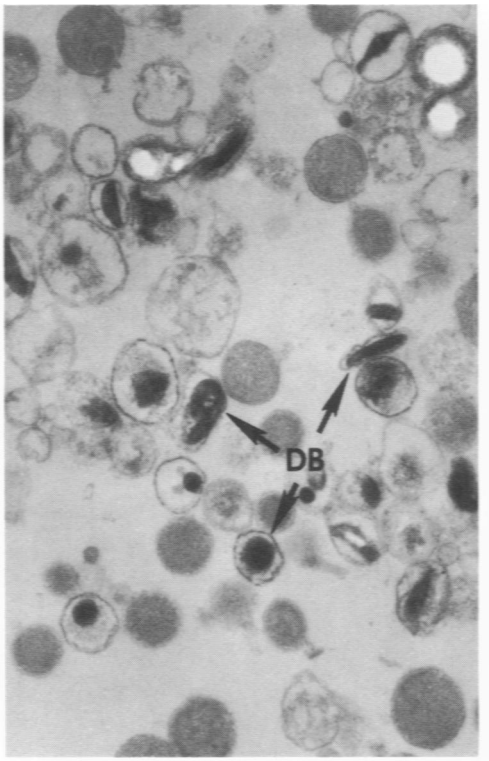
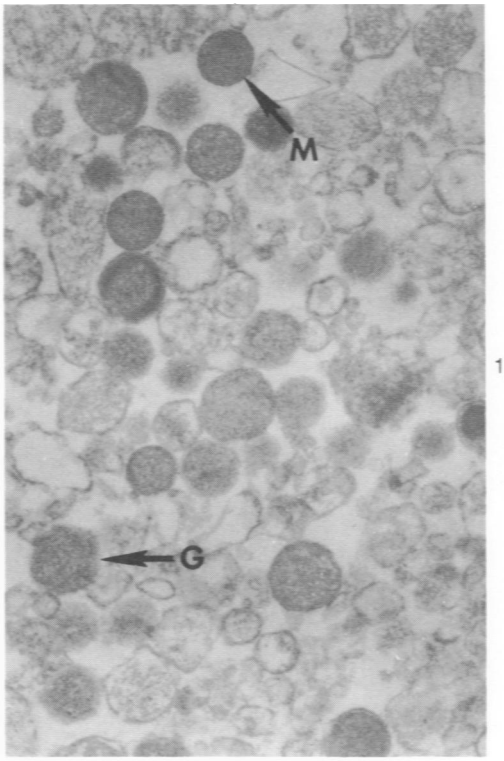
## Legends for Figures

**Figure 1A-C**—Subcellular platelet fractions. **A**—Washed platelets were frozen, thawed, sonicated, and centrifuged at 900*g* for 20 minutes to obtain a dense body (*DB*)-rich fraction. **B**—The supernatant over the first pellet was centrifuged at 13,000*g* for 20 minutes to obtain a granule (*G*)-enriched fraction. This fraction also contains mitochondria (*M*). **C**—The supernatant from the second centrifugation step was further centrifuged at 105,000*g* to obtain a microsomal fraction. (**A** and **B**, after staining with lead citrate and uranyl acetate, **C**, before staining; **A**, × 25,000; **B**, × 25,000; **C**, × 22,000)

**Figure 2**—A platelet from a sample of normal C-PRP incubated for peroxidase activity. The electron-dense peroxidase reaction product is specifically localized to the dense tubular system (*DTS*). This platelet and subsequent platelet sections shown in Figures 3–6 were not poststained with uranyl acetate or lead citrate so that the peroxidase reaction product can be seen clearly. Granules (*G*), dense bodies (*DB*), and mitochondria (*M*) can be identified within the platelet. (× 24,000)




1A

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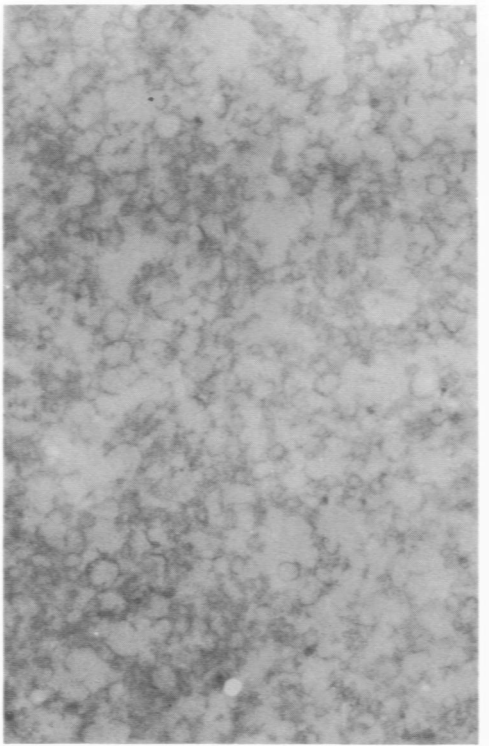
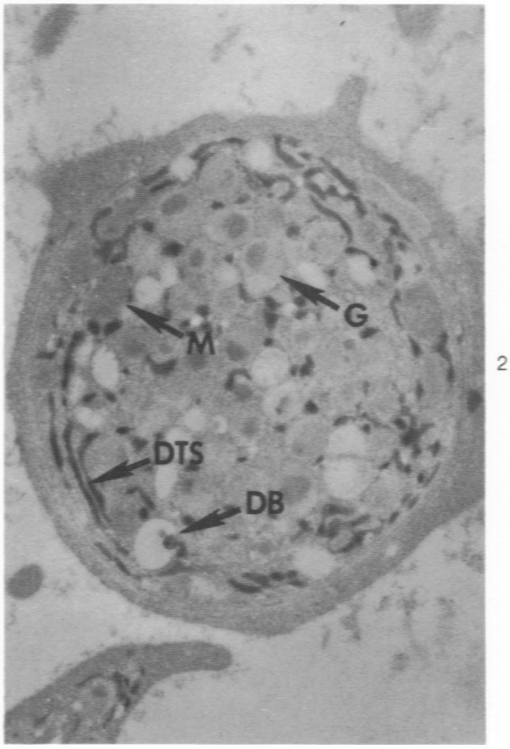
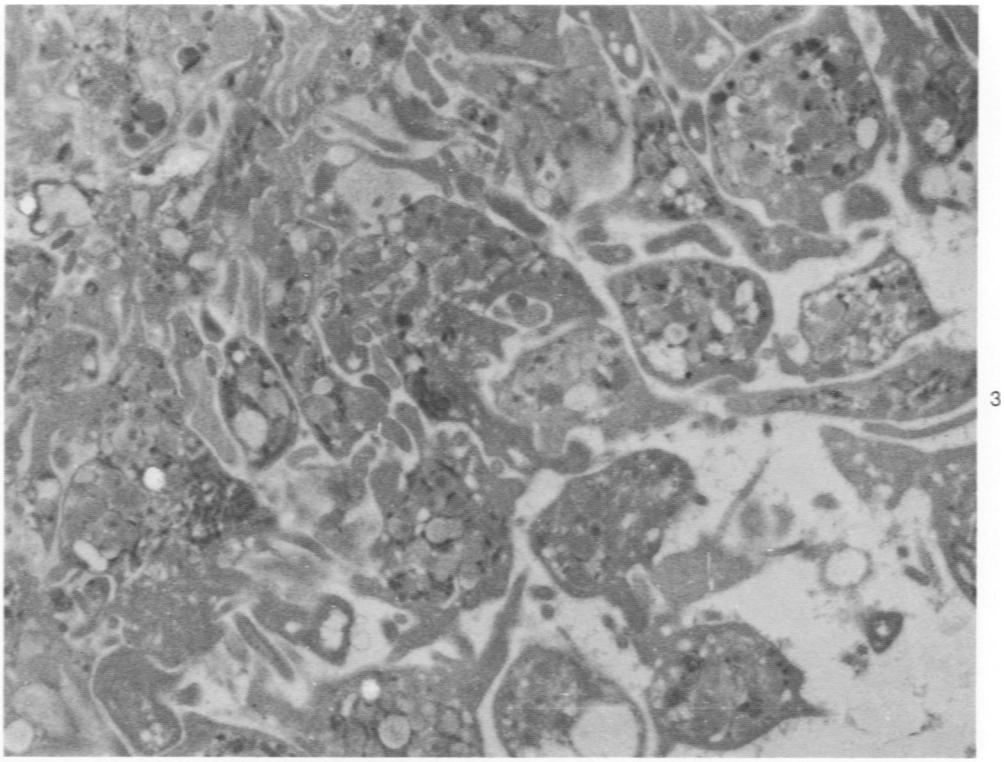


1C




2

**Figures 3 and 4**—C-PRP incubated for 30 minutes with 0.01 M aminotriazole and then stirred with collagen. A large aggregate is visible in **3** and considerable peroxidase activity can be seen associated with the platelet dense tubular system (DTS). A higher power view of platelets from this sample shown in **4** reveals internal transformation (*arrow*) within a platelet inside the aggregate. (**3**, × 5500; **4**, × 17,400)



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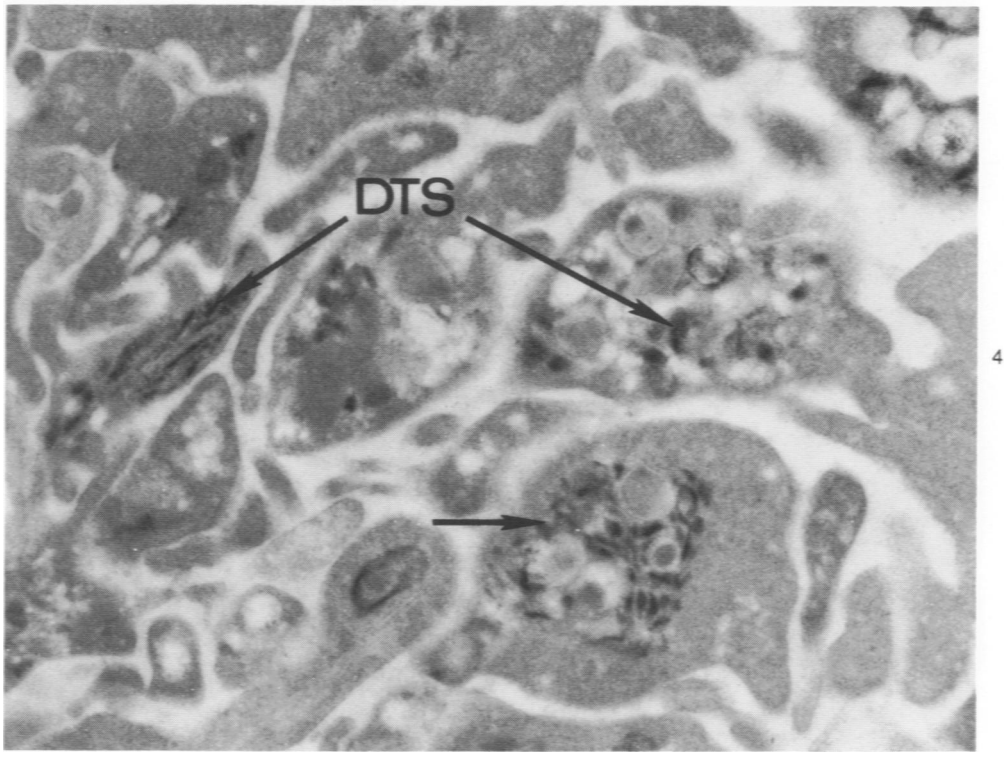


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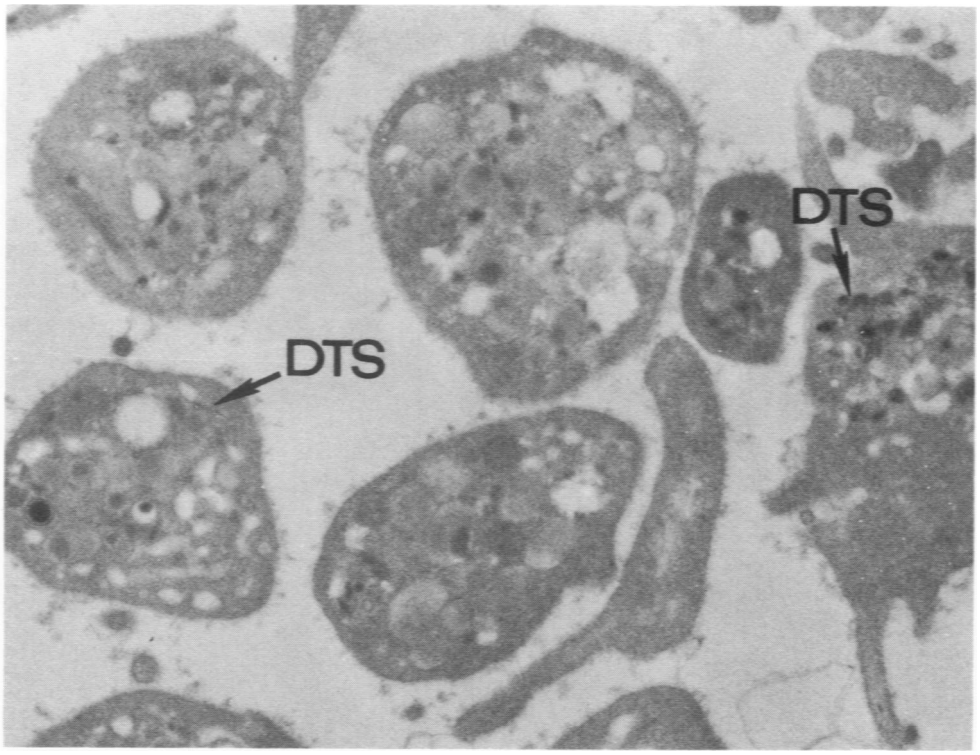
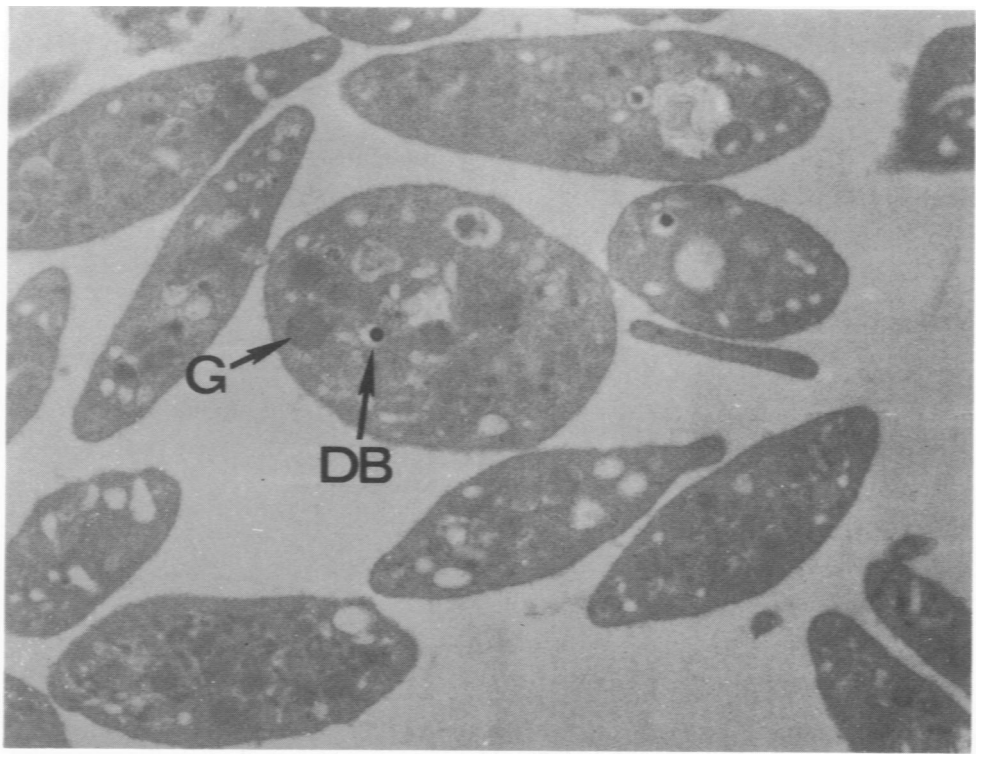



5




6

**Figure 5**—Platelets from a sample of C-PRP incubated for 30 minutes with 0.02 M aminotriazole, stirred with collagen, fixed, and stained for peroxidase. Platelets reveal various stages of shape change and pseudopod formation. The peroxidase reaction associated with the dense tubular system (*DTS*) is less intense than in the previous two illustrations. (× 21,000) **Figure 6**—Platelets from a sample of C-PRP incubated for 30 minutes with 0.05 M aminotriazole, stirred with collagen and then fixed and stained for peroxidase. Platelets have retained their resting discoid shape, with granules (*G*) and dense bodies (*DB*) distributed randomly throughout the cytoplasm. No peroxidase activity is visible within the dense tubular system. (× 17,000)