for by the greater fecal loss in the D_2 and nonsupplemented animals. This is most likely the result of the failure to reabsorb calcium excreted via the intestine in these two groups. Urinary loss of Ca^{47} was significantly different in the three groups but the levels were so low in all three groups (0.31 percent in the D_3, 1.38 percent in the D_2, and 3.41 percent in the nonsupplemented) that it appears that if this reflects any action of vitamin D_3 or D_2 or both at the level of the kidney it is not of great importance in maintaining calcium balance. The differences in retention clearly indicate differences in deposition in the bone. However, neither the estimation of activity of the serum nor the external counting over the head are sufficiently accurate to demonstrate the differences in the degree of retention observed after intravenous injection. Whether vitamin D_2 or D_3 has an active role at the level of the bone was not demonstrated by this study and warrants additional experimentation.

The data for both oral and intravenous administration of Ca^{47} demonstrate a primary effect of vitamin D_3 on intestinal absorption of calcium, rather than at the level of the bone or kidney, and clearly indicate that vitamin D_3 is more active than vitamin D_2 in promoting calcium absorption in Cebus monkeys. Further investigation of the effectiveness of various forms of vitamin D in man is warranted in light of this evidence in a primate species.

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Ultrastructure of Thrombosthenin, the Contractile Protein of Human Blood Platelets

Abstract. *Partially purified thrombosthenin with adenosine triphosphatase activity similar to that of actomyosin was subjected to electron microscopy. More than 50 percent of the material consisted of fibrils 80 to 100 angstroms in width. Occasional fibrils suggested a periodic structure. The morphology of isolated thrombosthenin resembled that of microfibrils in the cytoplasm and pseudopods of intact platelets.*

It has been known for almost a century that blood platelets are contractile and that they play an essential role in the process of clot retraction. Although this phenomenon has been observed and studied in many laboratories, the underlying mechanism is still not completely understood. In 1959 Bettex-Galland and Lüscher (*1*) extracted a contractile protein, thrombosthenin, from human platelets. Like muscle actomyosin, the activity of this protein depended on the presence of adenosine triphosphate (ATP) and Ca^{++} ions. It was, therefore, postulated that its muscle-like adenosine triphosphatase activity might be responsible for platelet-dependent clot retraction. These observations were confirmed by others (*2*, *3*). Thrombosthenin has been partially purified (*3*), and thus analysis of its ultrastructure has been facilitated.

We now describe the electron-microscopic appearance of isolated thrombosthenin and the correlation of its morphology with that of subcellular structures observed in intact platelets. Platelets were collected from 5 to 10 liters of human blood, washed six times in a solution of 0.1*M* sodium citrate and 0.15*M* NaCl, and suspended in 0.6*M* KCl in 0.015*M* tris (hydroxymethyl)aminomethane (tris) buffer at *p*H 7.5. They were gently lysed with *n*-butanol according to the procedure of Grette (*2*). The debris was sedimented by centrifugation at 12,000*g* for 15 minutes, and the contractile protein contained in the supernatant was precipitated with 0.002*M* $MgSO_4$ at 4°C. Centrifugation at 8700*g* for 10 minutes yielded a stiff gel. This gel was dissolved with 0.6*M* KCl in tris buffer and reprecipitated with 0.002*M* $MgSO_4$ six times. It was then further purified by chromatography on Bio-Gel P-300.

The chemical and immunologic properties of this preparation have been described in detail (*3*) and will only be summarized here. The adenosine triphosphatase activity of isolated thrombosthenin was shown in the presence of $5 \times 10^{-4}M$ ATP; 1 mg of the protein released 3 μg of in-

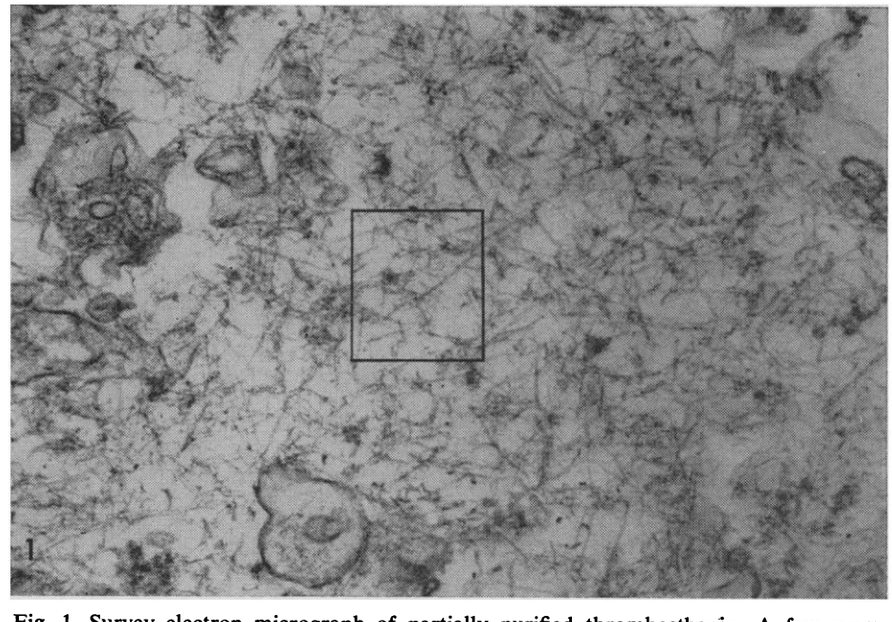

Fig. 1. Survey electron micrograph of partially purified thrombosthenin. A few membranes and amorphous material contaminate this preparation, which consists mostly of fibrillar structures (× 51,000). Inset seen at higher magnification in Fig. 2.

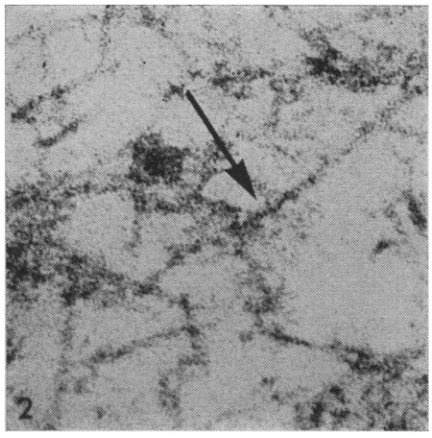

Fig. 2 (left). Higher magnification of inset in Fig. 1. Arrow points to fibril which appears to have a periodic structure (× 164,000).

organic phosphorus over a period of 30 minutes. This activity could be abolished with Mersalyl but it could only be inhibited to a limited extent with ouabain; this difference distinguishes it from membrane adenosine triphosphatase active in "ionic pumps." Addition of ATP resulted in a marked decrease in relative viscosity comparable to that obtained with actomyosin extracted from smooth muscle. The isolated protein showed the superprecipitation phenomenon when ATP was added. On immunoelectrophoresis against a specific rabbit antiserum, thrombosthenin yielded a single precipitin line in the β-globulin region. The antiserum to thrombosthenin interfered with clot retraction mediated by normal platelets.

In the sedimented form, the protein was suitable for fixation and embedding by standard procedures used in electron microscopy. It was fixed in 3 percent glutaraldehyde (*4*), or 2 percent osmium tetroxide (*5*), or both. It was embedded in Epon 812 (*6*). Thin sections were cut with an LKB ultrotome and viewed with a Siemens Elmiskop I electron microscope at instrument magnifications ranging from 10,000 to 60,000.

Survey electron-micrographs at low magnification (Fig. 1) showed that about 50 percent of the material consisted of fibrils. The remainder comprised membrane fragments, amorphous protein, and particles. At higher magnification (Fig. 2), the fibrils ranged between 80 to 100 Å in width. Their length was difficult to determine since they crisscrossed into and out of the plane of section. The appearance of occasional fibrils suggested a periodic structure. In cross section the fibrils appeared as irregularly shaped dots. Identical fibrils can be resolved throughout the cytoplasm of intact platelets. These are particularly striking with pseudopod-like projections, in which the microfibrils become oriented in the same direction as the pseudopod (Fig. 3).

Comparison of Fig. 1 with Fig. 3 demonstrates the resemblance of microfibrils to the fibrils of isolated thrombosthenin. It is noteworthy that the morphology of platelet microfibrils is very similar to that of myofibrils in human smooth muscle, and that "primitive muscle" has been found in other mammalian cells (*7*). These findings raise the question whether platelet microfibrils may indeed represent myofibrils. It is, therefore, possible that the microfibrils are the morphologic counterpart of the biochemically and immunologically characterized contractile protein, thrombosthenin. Definitive identification must await complete purification of the protein.

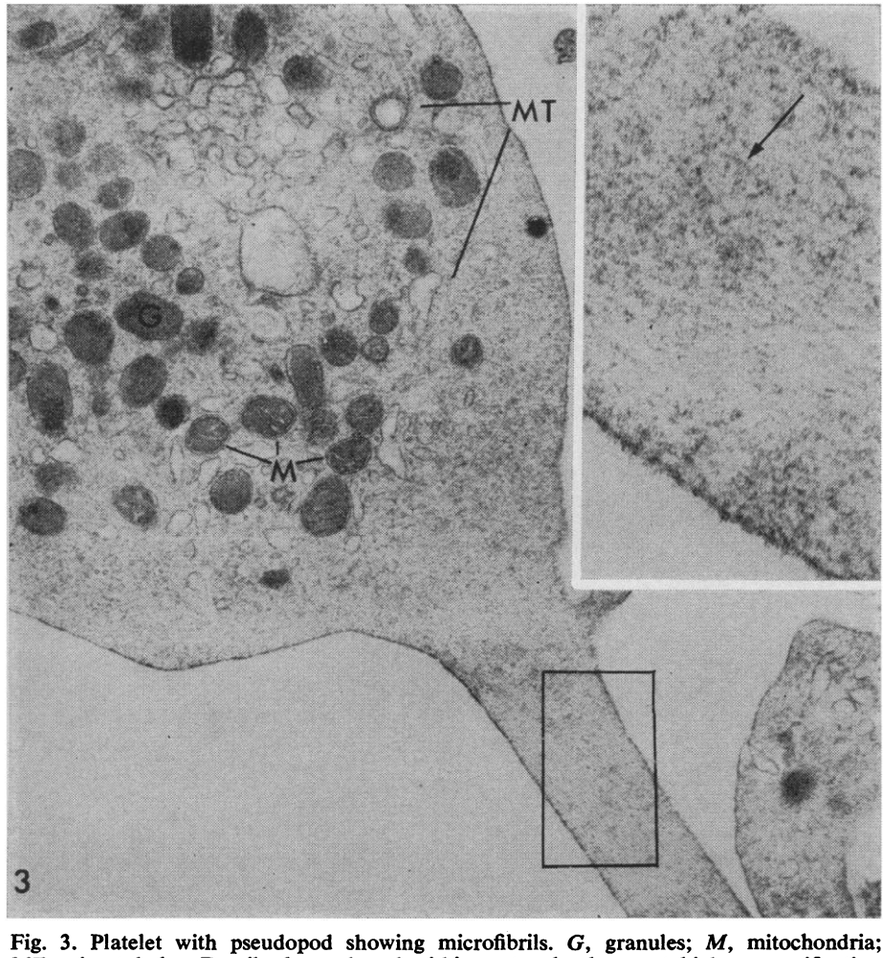

Fig. 3. Platelet with pseudopod showing microfibrils. *G*, granules; *M*, mitochondria; *MT*, microtubules. Detail of pseudopod within rectangle shown at higher magnification in upper right-hand corner. Arrow points to microfibril which appears to have periodicity (platelets, × 24,000; detail, × 108,000).

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