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The Influence of Prostaglandin Endoperoxides on Platelet Ultrastructure

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Prostaglandin endoperoxides produced during aggregation of blood platelets are potent promoters of platelet aggregation and the release reaction. The present investigation has studied the effects of prostaglandin endoperoxides produced by platelet microsomes after incubation with arachidonic acid on the ultrastructure of platelets. Prostaglandin endoperoxides caused platelet pseudopod formation and internal transformation associated with a contractile wave within the platelet. The contractile process was similar to that seen following incubation of platelets with collagen or thrombin but was more complete than that seen with 25 μM ADP. Platelet aggregation was more prominent in unstirred samples incubated with the prostaglandin endoperoxides than in samples similarly incubated with 25 μM ADP. Dilatation of the open canalicular system was not a prominent feature except at 45 minutes after addition of the endoperoxides to the platelets, when the platelets appeared to be in a recovery phase. We conclude that the endoperoxides promote platelet stickiness and platelet aggregation by directly or indirectly stimulating the platelet contractile system. (Am J Pathol 80:189–202, 1975)

THE PROSTAGLANDIN ENDOPEROXIDES, PGG_2 and PGH_2, which are synthesized in platelets in response to aggregating agents, have been found to be potent triggers of platelet aggregation.[1-5] Furthermore, it has been shown that the endoperoxides act as intercellular messengers promoting platelet stickiness and the release reaction.[6] Previous investiga-

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tions have shown that agents which induce platelet secretion may operate by triggering a contractile wave within the platelets [7-9] or may initiate dilatation of the open canalicular system as occurs following exposure of platelets to phorbol myristate acetate.[10,11] Finally, it is possible that the release reaction which occurs following exposure of platelets to prostaglandin endoperoxides may result from platelet damage. The present investigation was undertaken to distinguish between these three possibilities by evaluating the action of the prostaglandin endoperoxides on platelets at the ultrastructural level.

Materials and Methods

The procedures used in this laboratory to obtain blood from normal donors, 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 (approximately 3×10^8 platelets/ml) to various drugs and experimental conditions, and prepare samples for transmission electron microscopy have been described in recent publications.[6-8,12] The prostaglandin endoperoxides were prepared using a modification of the method of Willis.[13] Suspensions of washed platelets were frozen, thawed, sonicated, and centrifuged at 13,000*g*. The supernatant was then centrifuged twice at 105,000*g* to obtain a final pellet of platelet microsomes. Fifty micrograms of arachidonic acid were then incubated for 45 seconds with a platelet microsomal preparation (protein concentration about 4 mg/ml, total volume of the incubation mixture 0.1 ml) prior to addition of 0.9 ml C-PRP, as described earlier.[6] Samples of C-PRP exposed to endoperoxides in this fashion were incubated without stirring at 37 C for intervals of 2, 5, 15, and 45 minutes prior to fixation. Controls included addition of C-PRP to: a) platelet microsomes alone, b) arachidonic acid alone, c) PGE_2, and d) $PGF_{2\alpha}$. To compare the early changes in internal transformation in platelets exposed to prostaglandin endoperoxides with the early changes induced by ADP, samples of C-PRP were incubated with 25 μM ADP at 37 C for 2- and 5-minute intervals. Results are based on data from experiments on 17 samples of blood from 9 different donors.

Results

Normal C-PRP and C-PRP Exposed to Platelet Microsomes, Arachidonic Acid, PGE_2, and $PGF_{2\alpha}$

Control platelets showed a discoid configuration with randomly placed organelles, granules, dense bodies and mitochondria, and bundle of microtubules in the plane of the largest platelet diameter just beneath the cell membrane (Figure 1). Platelets incubated for up to 60 minutes at 37 C showed essentially no morphologic changes. Examination of platelets which had been exposed to 50 to 1000 ng/ml PGE_2 or PGF_2 or to 50 μg/ml arachidonic acid revealed no significant differences from the control platelets. Platelets incubated with microsomes generally retained their discoid form though in some preparations, minor degrees of pseudopod formation and a slight dilataion of the open canalicular system were visible (Figure 2).

Influence of Prostaglandin Endoperoxides on Platelets

Changes observed following addition of C-PRP to the prostaglandin endoperoxides depended on the duration of incubation. Marked changes in the platelets were apparent at 2 minutes. Platelets lost their discoid form, extended pseudopods, and rearranged their internal contents. The degree of internal transformation varied from platelet to platelet. Some platelets retained most of their granules which had been moved centrally and were closely surrounded by the microtubule bundle (Figure 3). In other platelets, the contractile wave had progressed until only one or two granules were left in the center of the microtubule ring (Figure 4). In occasional platelets, only a mass of contractile gel remained in the center of the degranulated platelet (Figure 5). Finally, some of the platelets showed a complete absence of granules, with only broken fragments of microtubules and elements of the dense tubular system remaining in the center of the platelet (Figure 6). In addition to single platelets, there were a variable proportion of the platelets that had formed small aggregates. The aggregates contained platelets showing changes similar to those observed in single cells. However, it was noticeable that, within aggregates of a few platelets, the shift of granule contents was towards the center of the aggregate rather than the center of each platelet (Figure 7).

At 5 minutes following exposure of the platelets to the endoperoxides, small aggregates had formed, with few platelets remaining single and unassociated with other cells. Larger aggregates characteristic of stirred samples of C-PRP were not seen. Platelets within these small aggregates were densely packed. The peripheral platelets were usually devoid of granules while the central platelets often had some intact granules. At 15 minutes following exposure of the platelets to the endoperoxides, the small, tightly packed aggregates were still visible, although this time the platelets within the center of the aggregate had also lost most of their granules (Figure 8).

By 45 minutes, the aggregates appeared to be breaking up. Some platelets appeared to have regained their discoid form, though most platelets were still irregular in shape with a variable number of pseudopods. The platelets remained largely degranulated and had by this time developed some dilatation of the open canalicular system (OCS) (Figure 9 and 10).

Platelets incubated for 2 or 5 minutes with 25 μM ADP showed internal transformation and pseudopod formation resembling samples of platelets incubated with the prostaglandin endoperoxides. However, it was rare for platelets incubated with 25 μM ADP to progress beyond the stage in which granules were concentrated in the cell center surrounded by the

microtuble ring as depicted in Figure 2. C-PRP incubated with 25 μM ADP also had fewer and smaller aggregates present at 5 minutes than did similar samples incubated with the endoperoxide.

Discussion

The present investigation has demonstrated that physical alterations in platelets produced by prostaglandin endoperoxides are similar to those caused by other agents such as thrombin, collagen, and epinephrine, which appear to exert their effects by triggering a contractile wave within the platelet.[7-9] Thus the initial changes seen following exposure of platelets to the endoperoxides consisted of shape change, pseudopod extension, internal transformation, and progressive central movement of platelet granules. As the contractile wave progressed, the granule contents were squeezed out of the platelet. Microtubules wound tightly around centrally grouped organelles gradually broke and split off from the circumferential band and penetrated into the pseudopods. At the end of the contractile wave a central mass of contractile gel or only a few broken microtubules and elements of the dense tubular system remained in the center of the degranulated platelet. As the internal changes progressed, the platelets became stickier, and small aggregates developed. These aggregates were very tightly knit at 5 to 15 minutes following addition of the endoperoxides; however, by 45 minutes these aggregates had started to break apart, and platelets began to show some return to their normal discoid form.

Though the initial changes of pseudopod extension and internal transformation caused by the endoperoxide were similar to those produced by incubation of platelets with ADP, there were important differences. After 5 minutes incubation of C-PRP with 25 μM ADP, the platelets rarely progressed beyond the early stages of internal transformation, and aggregates were smaller and less numerous. Since 25 μM ADP is in excess of the amount of ADP which can be released from 3×10^8 platelets, the result suggests that the changes visualized in the platelets following incubation with the endoperoxides were the direct effect of the endoperoxides and not just the effect of ADP released from the platelets.

The appearance of the platelets 2 minutes after addition of the endoperoxides did not reveal the marked dilatation of the open canalicular system observed following exposure of platelets to phorbol myristate acetate.[10] Aggregates seen at 5 and 15 minutes showed platelets which were predominantly closely packed without a markedly dilatated open channel system evident in platelets aggregated after stirring with phorbol myristate acetate. Only at 45 minutes did the platelets which had been ex-

posed to the endoperoxides show consistent dilatation of the open canalicular system. The dilatation appeared related to the relaxation cycle of the contractile wave, with aggregates breaking up into individual platelets manifesting various degrees of return toward their normal discoid shape. The ability of platelets to recover their normal configuration provides evidence that the prostaglandin endoperoxides do not produce release by causing platelet damage.

The results of the present investigation provide a basis for distinguishing between the three possible mechanisms, initiation of a contractile wave, dilatation of the open canalicular system, and cell injury, whereby prostaglandin endoperoxides might produce release of the platelet storage pool. The evidence presented demonstrates that the platelets undergo a contractile wave following exposure to prostaglandin endoperoxides to an extent which cannot be entirely explained by release of ADP from platelets. In contrast to the findings of Sedar *et al.*, our findings do not show an action of the prostaglandin endoperoxides on dilatation of the open canalicular system.[15] It is possible that the substantially larger concentration of arachidonic acid needed to produce aggregation when added directly to C-PRP in their study may have contributed to their findings. Finally no evidence of platelet damage was seen. We, therefore, conclude that the action of the prostaglandin endoperoxides is due to a direct or indirect triggering of the platelet contractile system.

The mechanism whereby the prostaglandin endoperoxides exert their effects on platelet contraction is uncertain. Experiments with the cation ionophore A23187 suggest that calcium flux may initiate similar changes to those seen following exposure of platelets to the prostaglandin endoperoxides [16,17] and all other aggregating agents capable of causing physiologic platelet section.[7-9] There is evidence to link the action of certain prostaglandins to calcium flux,[18,19] and it is possible that the endoperoxides trigger platelet contraction by this mechanism. The present investigation did not distinguish between the two prostaglandins PGG_2 and PGH_2. Further studies are planned to isolate the two endoperoxides to determine whether one or both of them are responsible for the changes in platelets demonstrated in this study.

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[*Illustrations follow*]

Figure 1—Discoid platelet sectioned in the equatorial plane. A circumferential band of microtubules (*MT*) lying just beneath the cell membrane supports the discoid shape of the cell. Granules (*G*), dense bodies (*DB*), mictochondria (*M*), and masses of glycogen particles (*Gly*) are distributed randomly in the cytoplasm. Channels of the open canalicular system (*OCS*) communicating with the cell surface and closed elements of the dense tubular system (*DTS*) are dispersed throughout the cell.

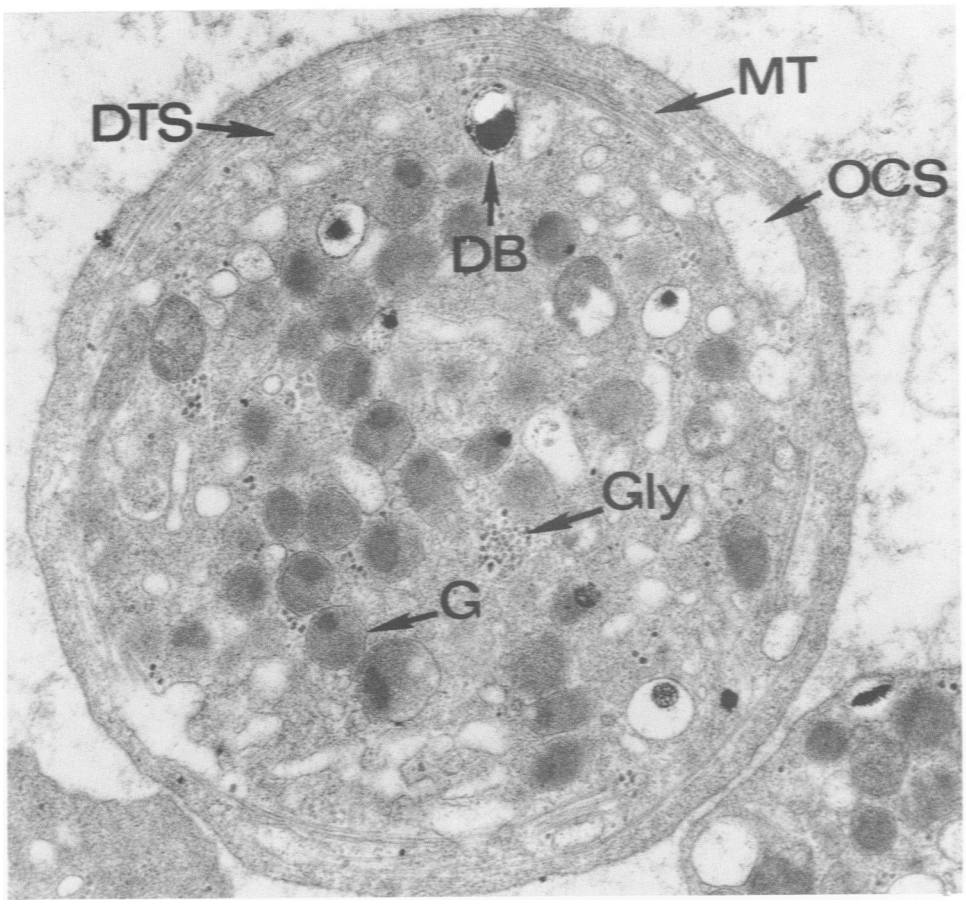
Figure 2—Platelets exposed to platelet microsomes (*Mic*) alone. The platelets are predominantly discoid in shape. Microtubules (*MT*) can be seen at the periphery of the cells, and granules (*G*) and masses of glycogen particles (*Gly*) are dispersed throughout the cell cytoplasm.

Figures 3-6—Sequential steps in the platelet contractile wave are illustrated in these four platelets. All platelets are from samples incubated for 2 minutes following addition of the prostaglandin endoperoxides. **3**—The surface contour of this platelet has become irregular with the projection of bulbous pseudopods. Granules (*G*), are clustered together in the center and surrounded by a close fitting band of microtubules (*MT*) and microfilaments (*MF*). **4**—The contractile wave has progressed in this cell, and most of the granules have been squeezed out of the platelet. One granule remains in the cell center surrounded by the microtubule ring which is showing evidence of breaking up, though it still surrounds the granule. **5**—In this platelet the contractile wave has progressed leaving a degranulated cell with a central mass of contractile gel. **6**—Another degranulated platelet in which broken fragments of microtubules and elements of the dense tubular system remain within the cell center.

Figure 7—A small aggregate in which the orientation of the contractile wave appears to be toward the center of the aggregate rather than toward the cell centers. The granules have moved toward the center of the aggregate rather than toward the individual cell centers. The platelets in this picture were incubated for 2 minutes after addition of the prostaglandin endoperoxide.

Figure 8—A tightly packed aggregate from a sample of C-PRP which had been incubated for 15 minutes with the prostaglandin endoperoxides. Cells are closely packed together and largely degranulated.

Figures 9 and 10—Dispersing aggregates seen in a sample of C-PRP which had been incubated for 45 minutes following addition of the prostaglandin endoperoxides. Cells are predominantly separated from one another, though most retain an irregular surface contour. One cell (*C*) can be seen which has reverted to a predominantly discoid configuration. Only a few granules (*G*) and dense bodies (*DB*) are retained in these platelets. Some dilatation of the open canalicular system (*OCS*) is present.



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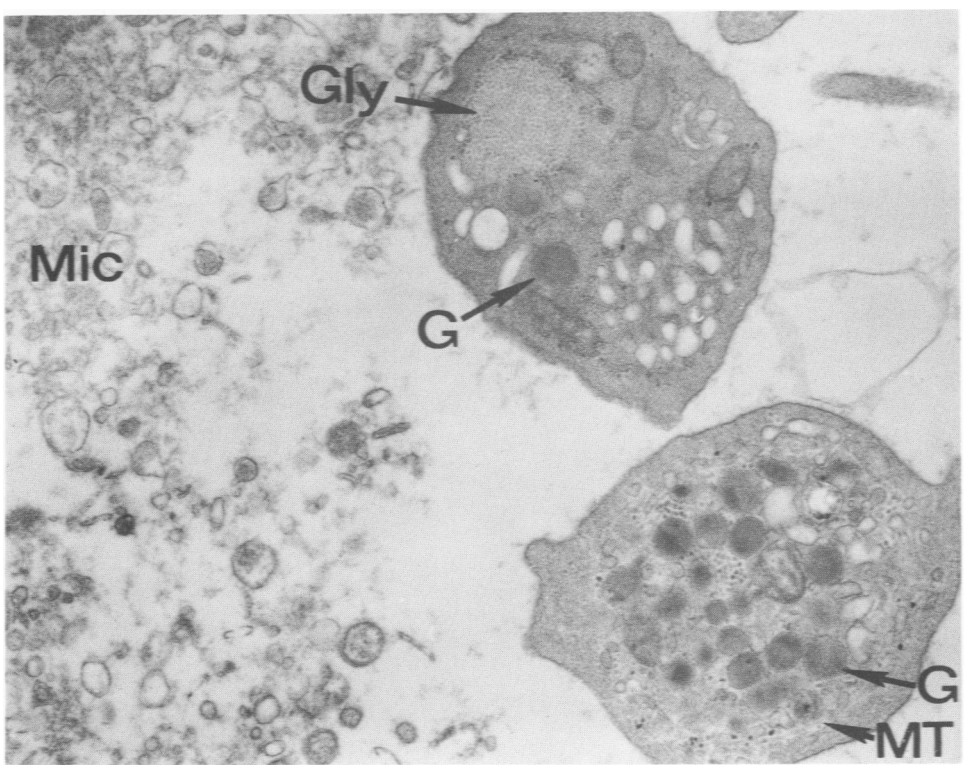

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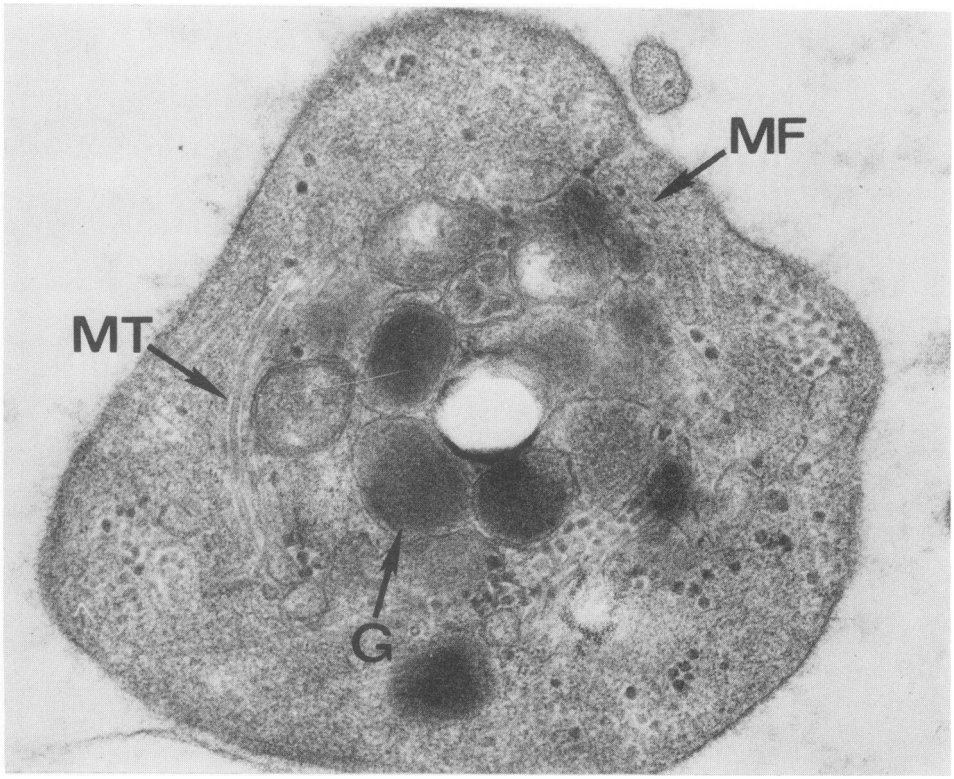

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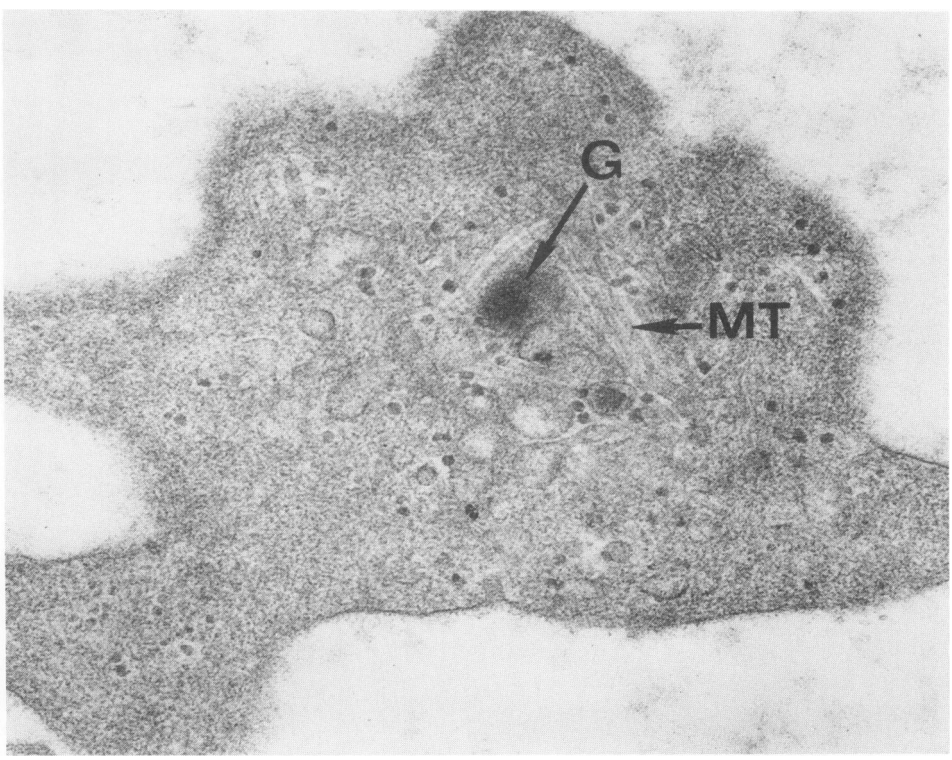

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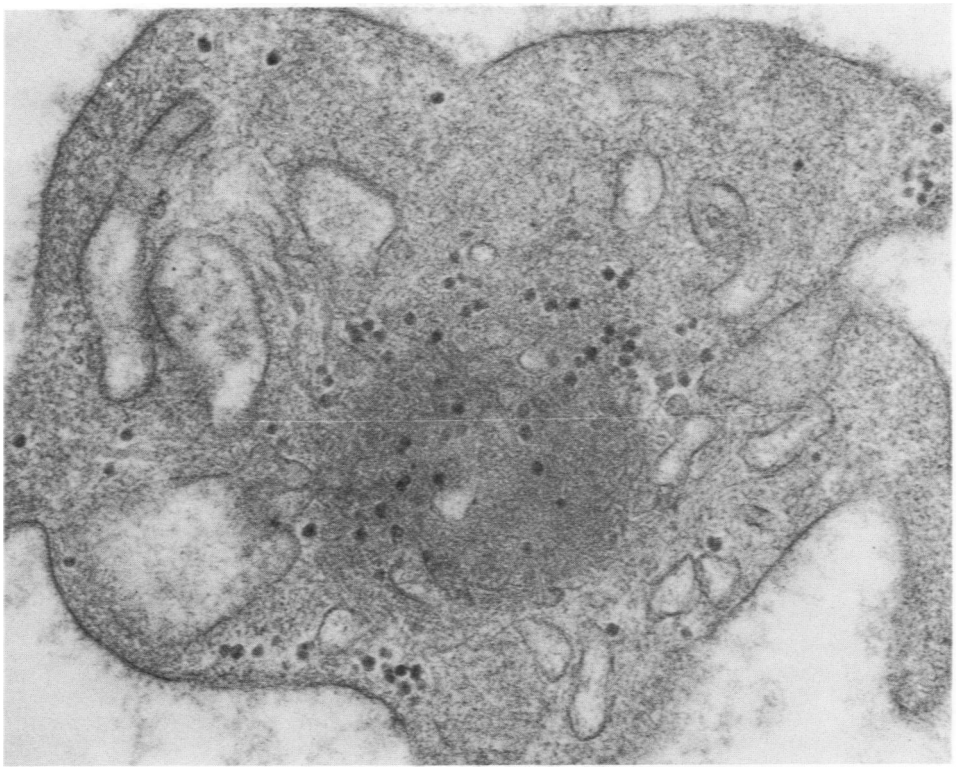

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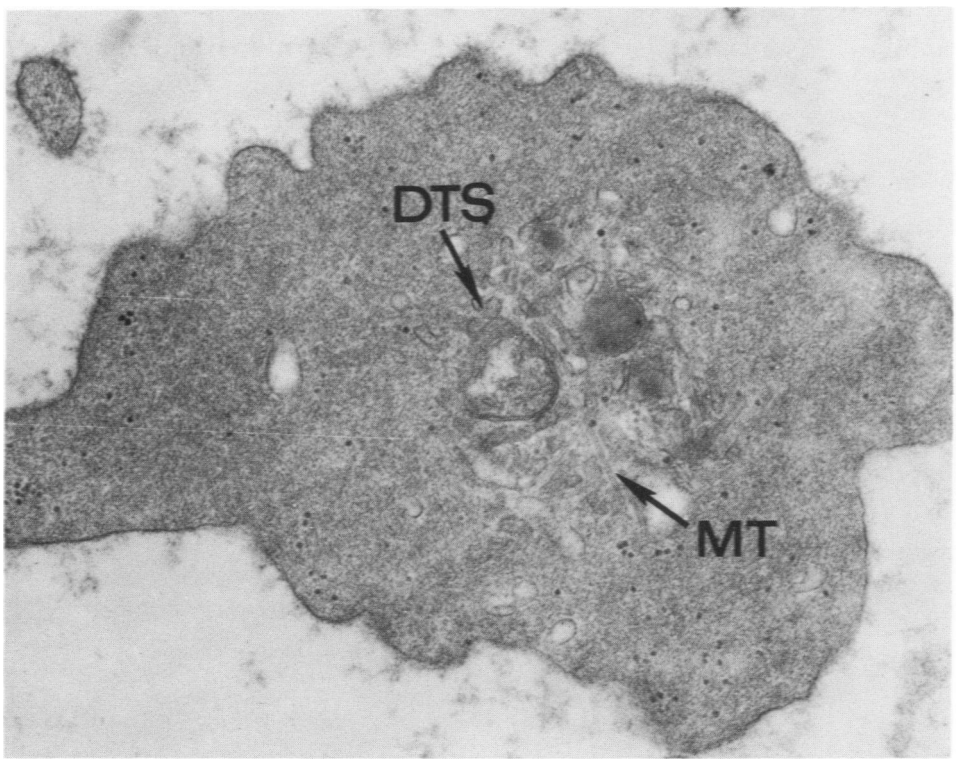

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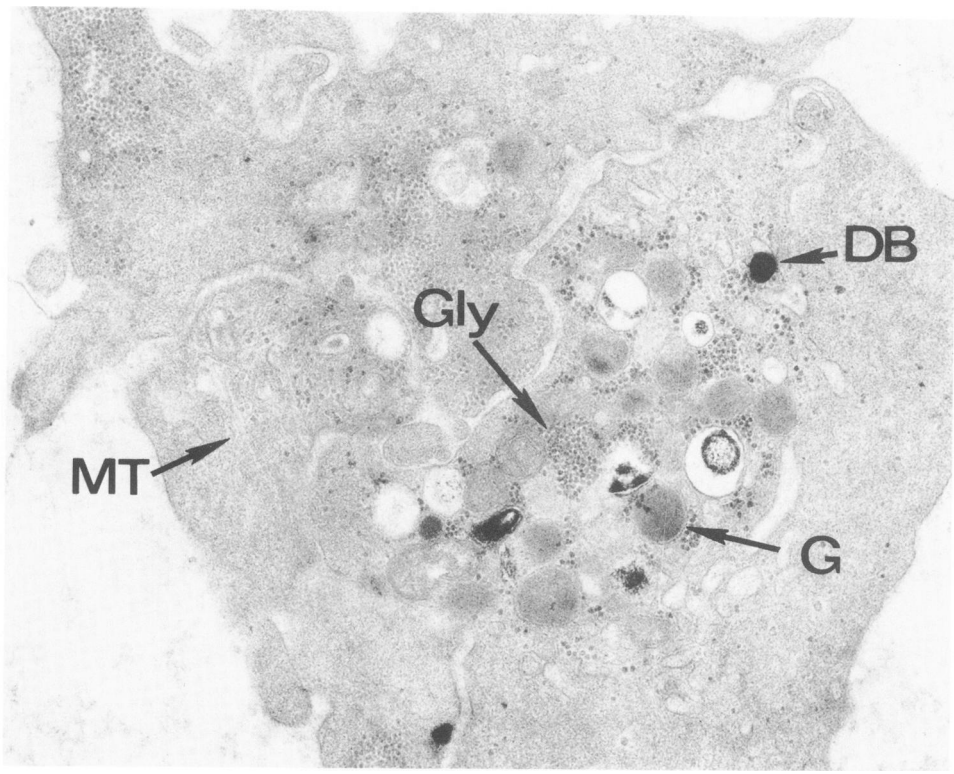

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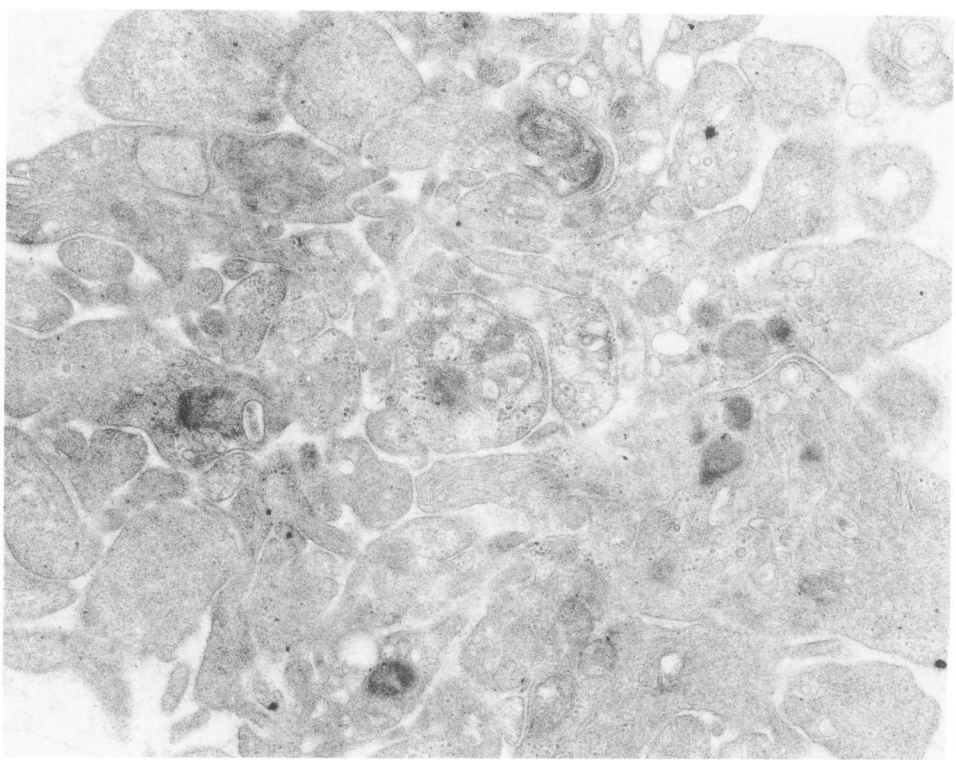

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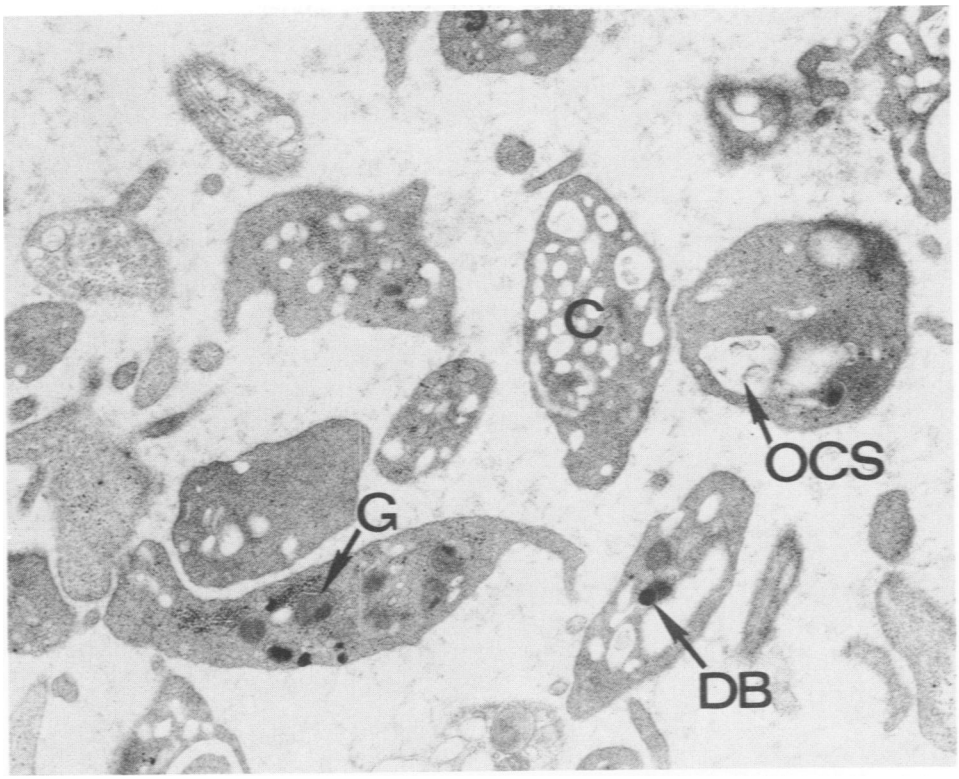

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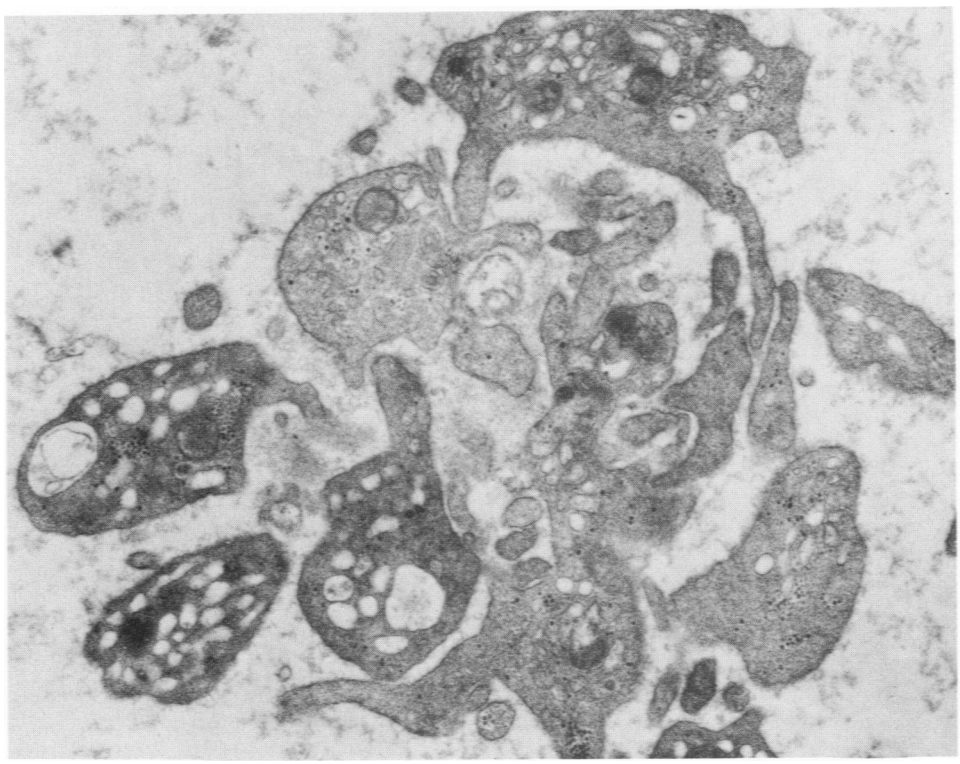

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