Interaction of Rabbit Platelets and Leukocytes in the Release of Histamine

Electron Microscopic Observations

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SEVERAL INVESTIGATORS [1] have studied the mode of allergic release of histamine from the platelets of rabbits that had been immunized against several different antigens. These studies on release of histamine required the presence of complement. Schoenbechler and Sadun [2] were the first to demonstrate that antigen-induced release of histamine from well-washed platelets of rabbits infected with *Schistosoma mansoni* necessitated only the inclusion of leukocytes from the infected animals. Recently, similar release of platelet histamine from rabbits immunized against protein antigens such as bovine serum albumin and horse spleen ferritin has been accomplished by adding these sensitized leukocytes.[3] The exact mode of histamine release from the platelets mediated either by the production of a soluble factor [3] from the activated leukocytes or by direct cell-to-cell interaction [4] remains unsettled.

This communication describes electron microscopic observations on the interaction between platelets and activated leukocytes in an attempt to shed light on the mechanism involved in histamine release from the platelets.

Materials and Methods

The preparation of leukocytes and of platelets from rabbits was described previously.[5] The procedures are outlined briefly as follows. The leukocytes from schistosome-infected rabbits were obtained relatively free of red blood cells by means of dextran agglutination. The major portion of the platelets from the leukocyte preparation were removed by differential centrifugation. Pure suspensions of lymphocytes were also made by employing a glass-bead column. The platelets used in this experiment were obtained from normal rabbits, and were essentially free of

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leukocytes. All experiments were conducted with leukocytes (sensitized) from infected rabbits and normal rabbit platelets washed 3 times with Tyrode's buffer to ensure the absence of free plasma. The buffer used throughout this experiment was Tyrode's solution. Four different combinations of the various cell suspensions of these platelets, leukocytes and antigen were made as follows:

Group	*Combination*
1	Normal platelets + sensitized leukocytes (control group)
2	Normal platelets + sensitized leukocytes + antigen
3	Normal platelets + antigen-activated leukocytes
4	Normal platelets + antigen-activated leukocytes + antigen

The activated leukocytes were made by incubating sensitized leukocytes with an equal volume of antigen. After incubation, the activated leukocytes were washed twice with Tyrode's buffer.

These four types of cell suspensions were fixed in 1.25% glutaraldehyde, 4% sucrose in 0.05 M phosphate buffer (pH 7.4). The fixed material was washed in 0.05 M phosphate buffer and postfixed in 1% OsO_4. The preparations were dehydrated in a series of ascending ethyl alcohols and propylene oxide and were finally embedded in Epon 812. The resulting blocks were cut with a Porter-Blum MT-2 ultramicrotome and were stained with 1% uranyl acetate and lead citrate. Sections from 10 blocks of each group were examined with a Siemens Elmiskop 101 electron microscope.

Observations

Morphology of Rabbit Platelets and Leukocytes

The morphology of the normal rabbit platelets will be described briefly since their ultrastructural characteristics are pertinent to this study. Rabbit platelets were elongated or ovoid in shape and possessed several cytoplasmic organelles (Fig 1 and 2). They contained mitochondria, large round-to-oval electron-opaque α granules, small "very dense" granules,[6] vesicles, microtubules, glycogen particles, endoplasmic reticulum and ribosomes. The α granules were abundant and measured 200–300 mμ in diameter. Each granule was surrounded by a unit membrane; the matrix, in general, was uniformly electron opaque and occasionally was separated from the surrounding unit membrane by a narrow translucent zone (Fig 2). The very dense granules were smaller than the α granules and measured about 200 mμ in diameter. They often appeared as round vesicles in which a very dense particle of 100 mμ was situated eccentrically. On rare occasions, the entire matrix was completely occupied with very electron-dense material.

The vesicles varied in shape and were limited by a unit membrane. The matrix was electron transparent and did not contain any stainable substance (Fig 2). The microtubules were usually located near the plasma membrane (Fig 2) and measured about 200 Å in diameter.

The leukocytes seen in these preparations were small lymphocytes,

monocytes, neutrophils and eosinophils. The small lymphocytes were round and contained a large, round nucleus, which occupied about 80% of the cell body (Fig 3). A narrow rim of cytoplasm surrounded the nucleus and contained several mitochondria, numerous ribosomes, microtubules and infrequent smooth endoplasmic reticulum. No rough endoplasmic reticulum was evident. The monocytes were larger than the small lymphocytes. The abundant cytoplasm and the presence of the rough-surfaced endoplasmic reticulum together with the larger cell size helped to differentiate the monocytes from the small lymphocytes. The granulocytes were round with irregular microvilli extending from their surface (Fig 1). The granulocyte cytoplasm contained a large number of electron-dense granules of various size and shape as well as several nuclei.

Interaction of Platelets and Leukocytes

In the suspension of platelets and sensitized leukocytes in Tyrode's solution without antigen (group 1), numerous platelets, leukocytes and erythrocytes were distributed randomly in the thin sections (Fig 1). There was no apparent interrelationship or close contact between the leukocytes and the platelets. No morphologic changes were observed in these cells.

Combinations of the platelets and sensitized leukocytes with addition of antigen (group 2) or of activated leukocytes and platelets without antigen (group 3) or with antigen (group 4) demonstrated remarkable changes in their physical relationship as well as their morphology. These changes were most pronounced in group 4 (Fig 4). There were many aggregates of platelets intermingled with leukocytes and cellular debris (Fig 4). The centers of these aggregates were composed of a few small lymphocytes and monocytes surrounded by numerous platelets. Many of these platelets were irregular in shape and possessed pseudopods (Fig 5) extending toward the centrally located lymphocytes and monocytes. Often these pseudopods were in close contact with these leukocytes and some protruded into the leukocytic cytoplasm at the base of the microvilli (Fig 8 and 9). Thus, the pseudopods of the platelets and microvilli of the lymphocytes and monocytes were interdigitated. The tips of the pseudopods of the platelets were often inserted into the cytoplasm of these leukocytes, forming a cytoplasmic anastomosis (Fig 10). No limiting membrane could be seen in these areas. Further morphologic changes were observed in these platelets. The cytoplasmic matrix of a platelet located near the leukocytes became more electron opaque than in the control group (Fig 8–11). The num-

ber of α granules decreased and the numbers of vesicles increased. The remaining α granules occasionally underwent changes in which their matrices became partially electron translucent (Fig 6). It appeared as if the matrix was in the process of discharging its contents. The very dense granules also decreased in number. The cell debris intermingled with these platelets often consisted of electron-opaque granules similar to the α granules and very dense granules (Fig 4 and 7). These changes appeared to be more prominent in the platelets in contact with the lymphocytes and monocytes than in those situated at the periphery of the cell aggregates. However, there were no lysed platelets observed in our preparations. The lymphocytes and monocytes appeared to maintain their morphology, with the exception of an increased number of microvilli. Although granulocytes were always present in the vicinity of these aggregates, their intermingling with platelets was minimal.

Discussion

It has been shown by several investigators [2–5] that histamine is released from the platelets in a suspension of sensitized leukocytes and platelets of rabbits when antigen is added to the suspension. Sensitized platelets of rabbits alone were unable to release histamine, even in the presence of antigen.[5] On the other hand, the activated leukocytes were capable of causing histamine to be released from rabbit platelets in the presence or absence of antigen. These findings indicate that activated leukocytes in some way interact with the rabbit platelets, causing histamine to be released regardless of prior activation. The present study clearly demonstrated that a physical interaction occurs between the activated rabbit leukocytes and normal rabbit platelets. Of particular interest is the formation of platelet pseudopods and the insertion of these pseudopods into the leukocytic cytoplasm with the concomitant decrease in number of platelet granular inclusions.

Although the fine structure of the rabbit platelets under various experimental conditions has been described by many workers,[6–9] the formation of pseudopods by the platelets during antigenic reaction has not been reported. Formation of pseudopods and their insertion into leukocytes indicates that cytotaxis of the platelets toward the activated leukocytes occurs in the presence of antigen. The factors controlling this cytotaxis is not obvious.

The close physical interaction between the sensitized leukocytes and the platelets noted in this study is not entirely in agreement with the findings of Henson.[3] He rarely observed an interplay between the sensitized leukocytes and the platelets when he studied preparations of

rabbit cells sensitized to bovine serum albumin and horse spleen ferritin. Since he did not regularly discern the association of the leukocytes and the platelets, he suggested that this contact was not necessary for the release of platelet histamine. Instead, he obtained a soluble factor from the reaction of platelets, antigen and sensitized leukocytes, which he found caused the release of histamine. Although Barbaro and Schoenbechler [4] were unable to obtain evidence for the production of a soluble factor, they have not ruled out the possibility of its existence. It is possible that both physical interaction of the platelets and sensitized leukocytes and a soluble factor from these leukocytes may be involved in the release of histamine.

Release of the platelet constituents under various conditions has been described.[10] Virus, bacteria, antigen-antibody complexes and nonbiologic particles coated with gamma globuin are reported to cause release of platelet contents. Similarly, the release of cytoplasmic organelles of platelets after interaction with sensitized leukocytes was reported by Henson,[3] and is likewise presently observed. Packham *et al* [10] reported that the mechanism by which platelets lose their constituents involved the formation of holes in the platelet membrane. They demonstrated lysed platelets by electron microscopy. Though the present authors did not observe lysed platelets, the loss of the plasma membrane at the tip of the pseudopods of the platelets after their insertion into the leukocytic cytoplasm may indicate the site from which platelet components leak out.

Although serotonin has been shown to be localized in the very dense granules,[11,12] a specific organelle site for the storage of histamine has not been established conclusively for the rabbit platelets. DePrada and his co-workers [12] fractionated rabbit platelets and separated the organelles by differential centrifugation. By this method, they found high activity of histamine in the layer predominately composed of the very dense granules and vesicles. Thus, they suggested that the histamine is present either in the very dense granules in association with serotonin or in the vesicles. On the other hand, since the morphology of the rabbit platelets (which are unique in that they contain large amounts of histamine) is not radically different from that of those animals containing small amounts of histamine, it is difficult to relegate histamine to any particular organelle. The decreased number of granular inclusions and the appearance of empty vesicles seen in this study may indicate that, under these experimental conditions, platelets release other constituents such as hydrolytic enzymes,[13] coagulation factor 3,[9] (both possibly located in α granules) and serotonin,[11,12] which is located in the very

dense granules. The increased number of empty vesicles observed in our study indicates that some of these may have originated from α granules and very dense granules, the matrices of which have been discharged. This is supported by the observation that free electron-dense granules similar to α granules and very dense granules are seen intermingled with the platelets.

The cell types that directly interact with platelets for histamine release have been disputed. Henson [3] suggested that the large mononuclear cells, possibly monocytes, are involved in the release of histamine from the platelets, while Schoenbechler and Barbaro [5] reported that small lymphocytes as determined by light microscopy are responsible for the release. In the present electron microscopic study, both small lymphocytes and monocytes are closely associated with the platelets. Although physical interaction was observed in the suspension containing platelets and activated small lymphocytes without monocytes, still the possibility cannot be eliminated that interaction between the platelets and monocytes is involved in histamine release.

Summary

The interaction between normal rabbit platelets and sensitized rabbit leukocytes activated with antigen was studied by electron microscopy in an attempt to elucidate the mode of histamine release from the platelets.

In the suspension of platelets, and sensitized leukocytes not activated with antigen (control), there was no physical interaction. In combinations of the platelets with sensitized leukocytes activated with antigen, remarkable changes in their physical and morphologic relationships were observed. Many aggregates of platelets surrounded small lymphocytes and occasional monocytes. Many of these platelets were irregular in shape with pseudopods extending toward the lymphocytes and monocytes. Often, the pseudopods were in close contact with these leukocytes and their tips were often inserted into the cytoplasm of these cells, forming an anastomosis. The platelets appeared to be vacuolated and to have lost some of their granular inclusions.

These findings indicate that the physical interaction between platelets and activated leukocytes, particularly small lymphocytes, plays an important role in the release of histamine from the rabbit platelets.

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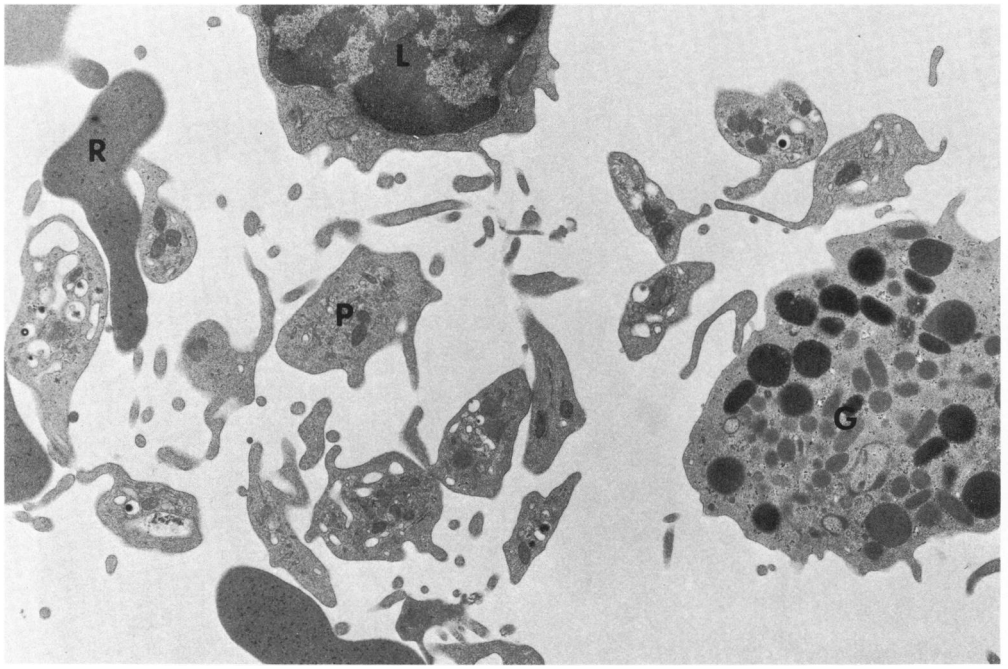
[*Illustrations follow*]



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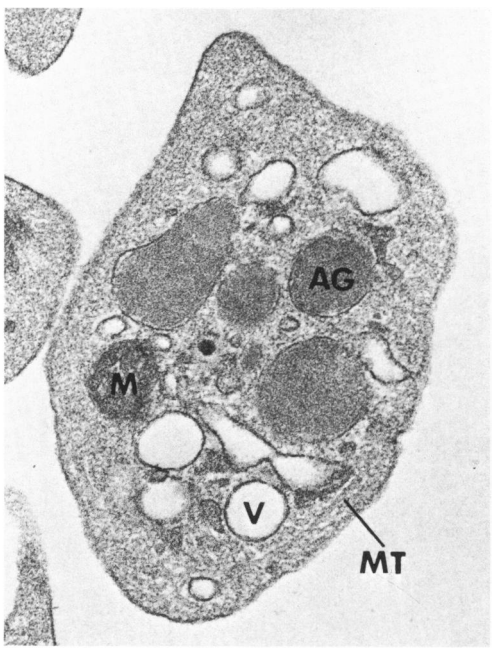

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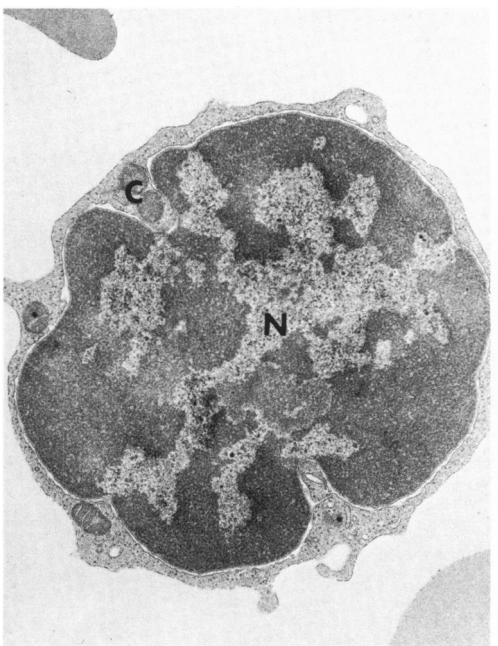

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Fig 1.—Cell suspension composed of platelets, and sensitized leukocytes of rabbits without the presence of antigen (group 1). Small lymphocyte (*L*), granulocyte (*G*) and erythrocytes (*R*) are intermingled with platelets (*P*). However, there is no physical interaction between these cells (× 12,000).

Fig 2.—Higher-magnification electron micrograph of platelet from control group. It possesses several electron-opaque α granules (*AG*), mitochondria (*M*), vesicles (*V*) and microtubules (*MT*). Very dense granules are not observed in this platelet (× 43,000).

Fig 3.—Small lymphocyte from group 1 containing large nucleus (*N*) surrounded by narrow rim of cytoplasm (*C*). Cytoplasm contains a few mitochondria and ribosomes. Endoplasmic reticulum is sparse (× 15,000).

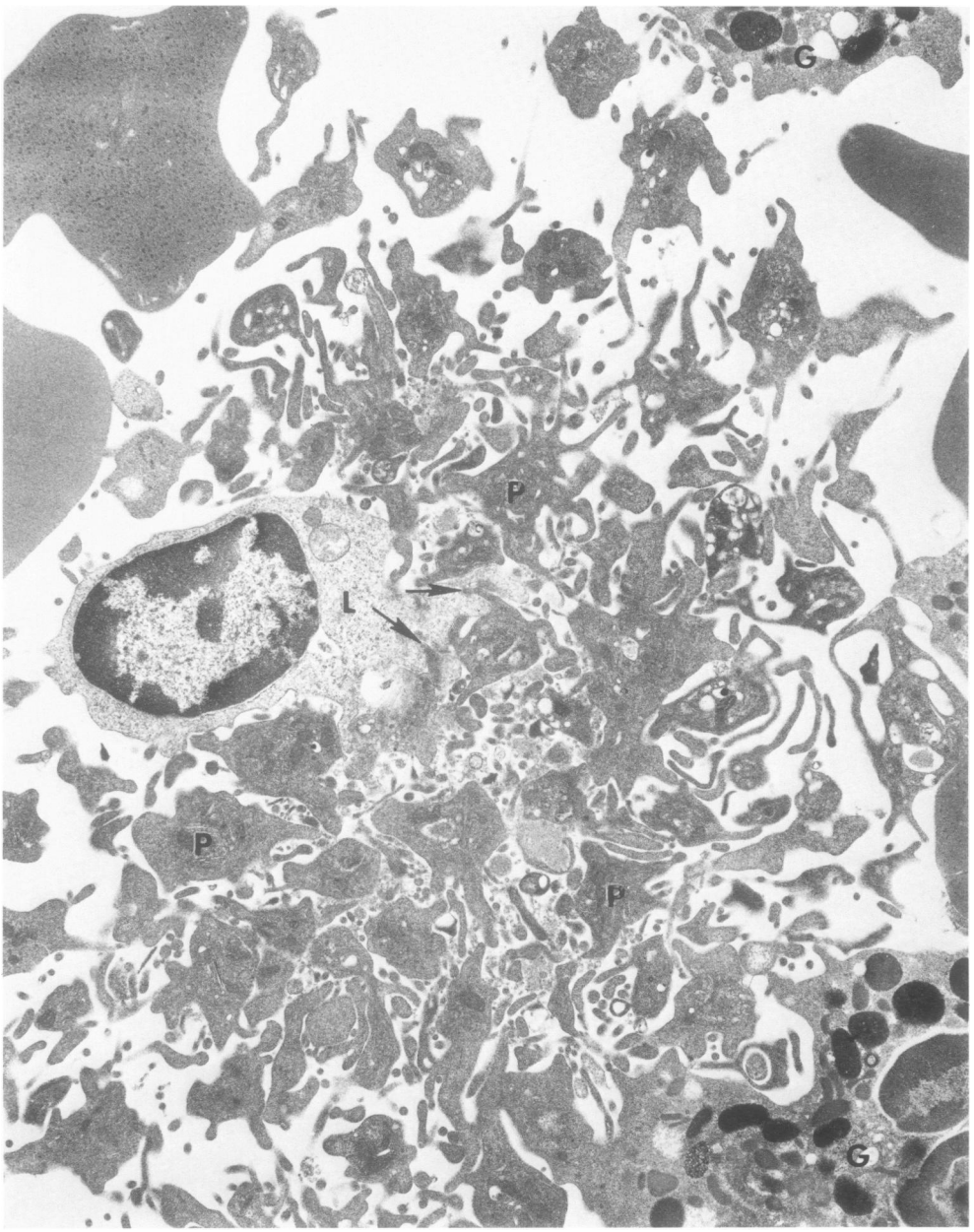
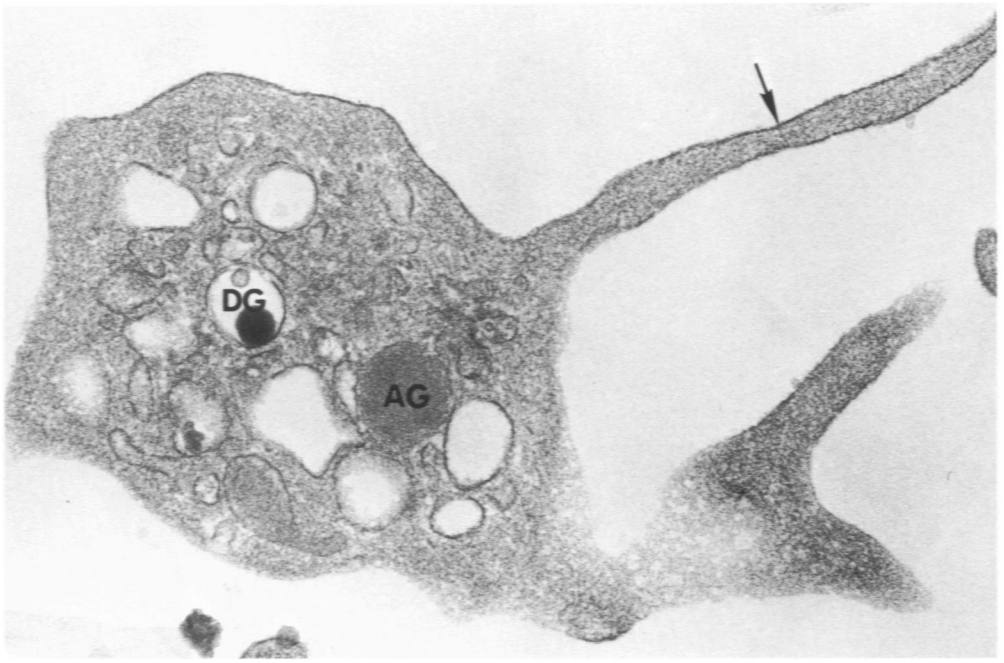


Fig 4.—Cell suspension consisting of platelets and activated leukocytes with added antigen (group 4). Aggregation of platelets (*P*) surrounds small lymphocyte (*L*). These platelets are intermingled with cell debris. Each platelet extends long pseudopods and some of them are in direct contact with small lymphocyte (*arrow*). A few granulocytes (*G*) are seen in periphery of this aggregate (× 10,000).



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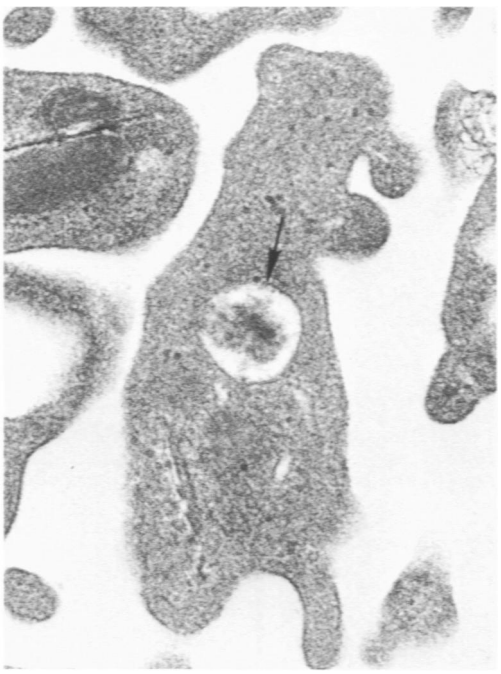

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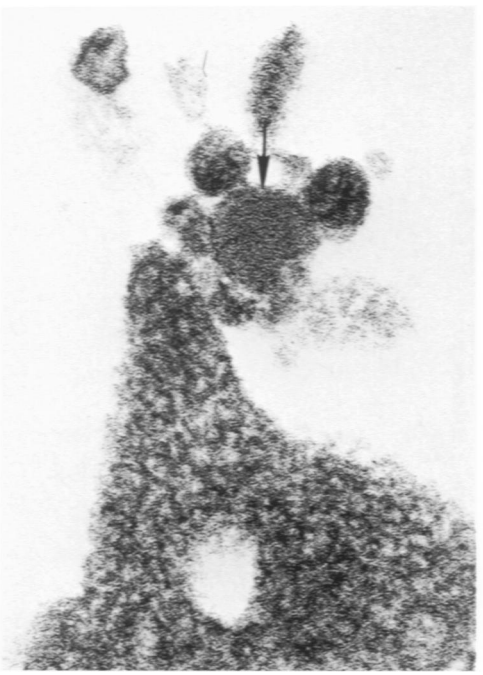

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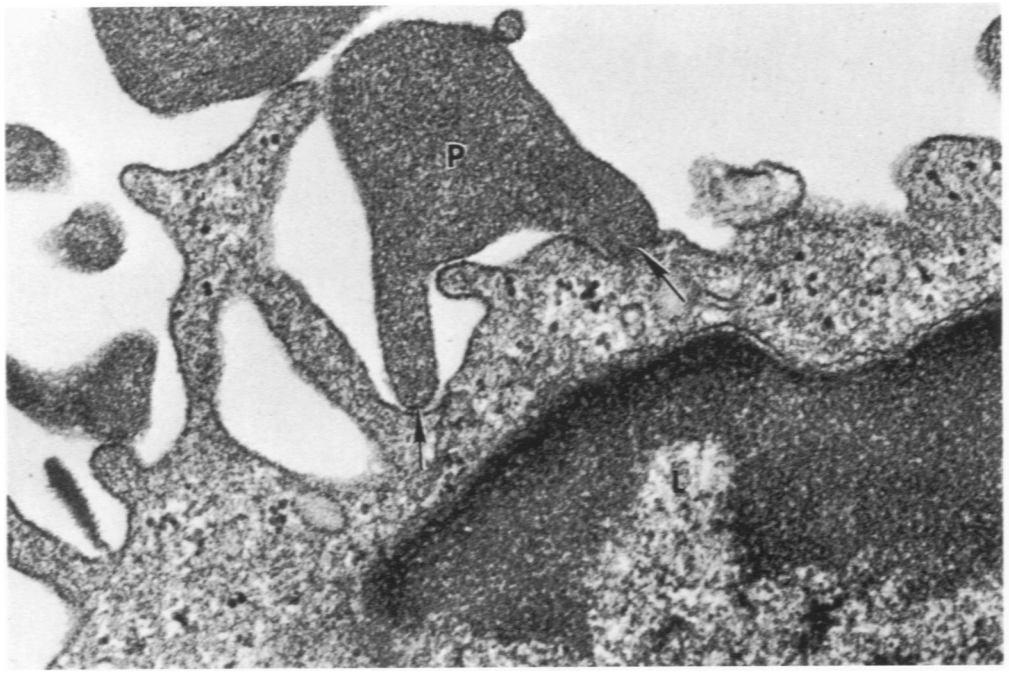
Fig 5.—Higher magnification photograph of platelet (from Group 2) with an extended long pseudopod (*arrow*). Platelet has many vesicles and vacuoles with two α granules (*AG*) and a very dense granule (*DG*) (× 46,000).

Fig 6.—Platelet with a vacuole (*arrow*) partially filled with electron-opaque material, the density of which is similar to that of the α granule (from group 3) (× 42,000).

Fig 7.—Globule (*arrow*) similar to α granule is observed outside platelet (from group 2) (× 60,000).

Fig 8.—Preparation consisted of platelets, activated leukocytes and antigen (group 4). Platelet (*P*) with two extended pseudopods is in direct contact (*arrow*) with cytoplasm of lymphocyte (*L*). Cytoplasmic organelles are absent within platelet (× 50,000).

Fig 9.—Another example (group 2) of physical interaction between pseudopods of platelets (*P*) and lymphocytic cytoplasm (*L*). Tips of platelet pseudopods insert into cytoplasm of lymphocyte (*arrow*). Cytoplasm of both cells merge, and that of platelet appears to vacuolate (*V*) (× 55,000).



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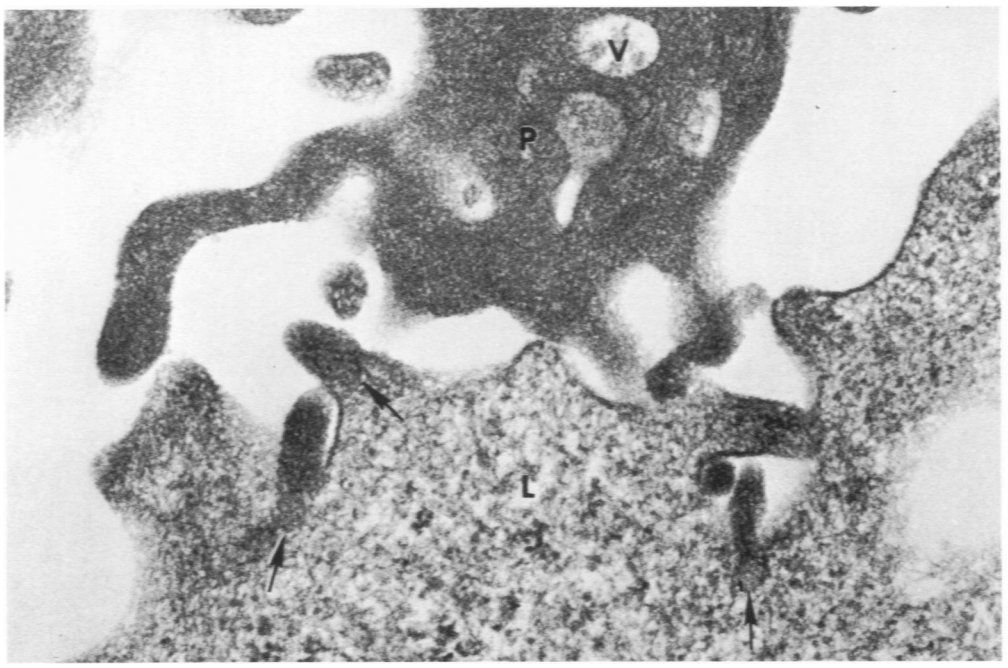

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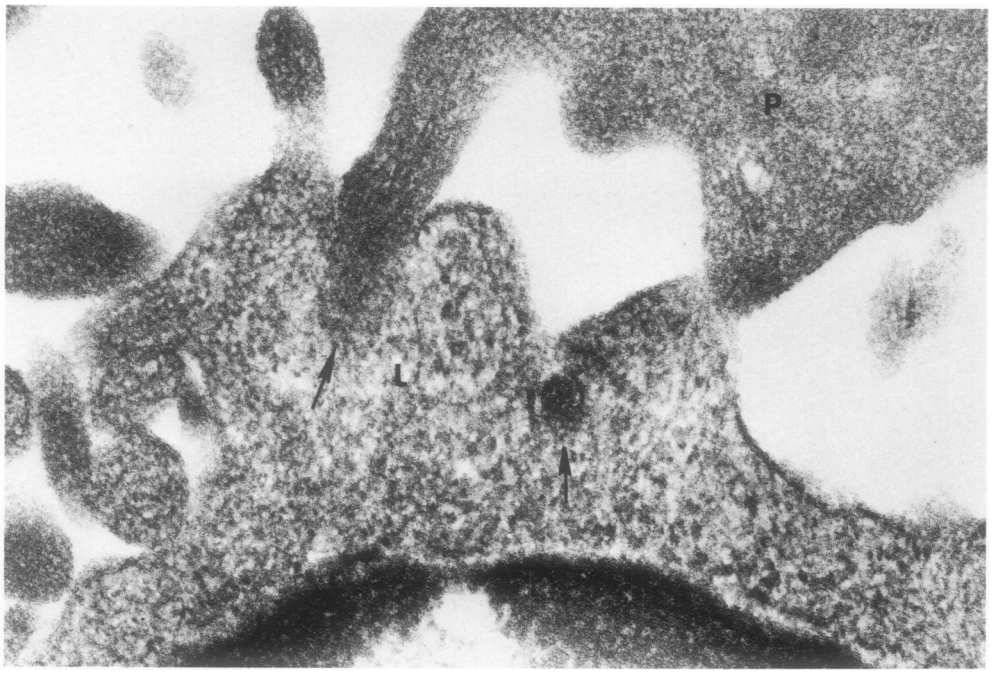

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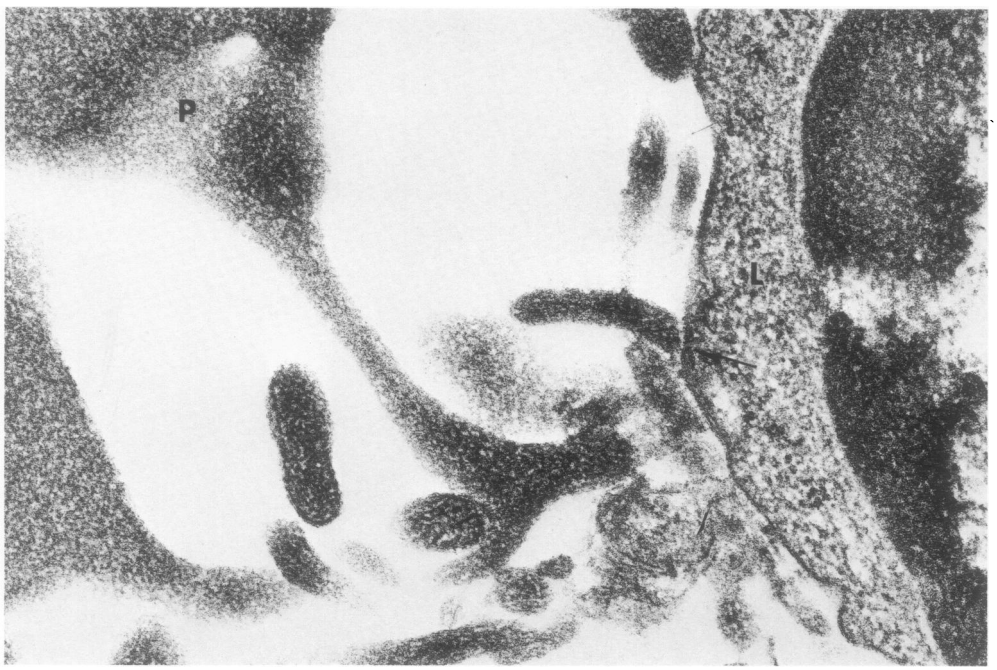

Fig 10.—Higher magnification micrograph demonstrating physical interaction between platelet (*P*) and small lymphocyte (*L*) (from group 4). Pseudopod tip of platelet inserts into the lymphocytic cytoplasm (*arrow*). Plasma membrane of both cells is not apparent at junction (× 71,000).

Fig 11.—Another example of interaction (*arrow*) of platelet (*P*) pseudopods and lymphocyte (*L*) (from group 3) (× 52,000).