Studies of Platelets in a Variant of the Hermansky-Pudlak Syndrome

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In 1959, HERMANSKY AND PUDLAK described two unrelated individuals with the triad of albinism, pigmented macrophages in the bone marrow and a hemorrhagic diathesis associated with prolonged bleeding time.[1] Despite mild bleeding symptoms, one of the original patients died after a subdural hematoma. Necropsy revealed that abnormal pigment, resembling ceroid, was deposited in macrophages of the reticuloendothelial system and endothelium of blood vessels in most organs of the body.[2] The absence of platelet and clotting factor abnormalities, and the presence of swollen, pigment-laden endothelium suggested that bleeding symptoms were due to spontaneous vascular rupture or pseudohemophilia.

Recently, other individuals and families with a similar clinical syndrome have been described whose platelets were found to be abnormal.[3-5] Maurer *et al* [4] observed intrinsically low levels and poor uptake of serotonin in platelets of patients, defective availability of ADP-induced platelet factor 3, and, by electron microscopy, the absence of platelet dense bodies. Two patients evaluated by Mills and Hardisty [5] had similar platelet defects. In addition, they found a marked decrease in the nonmetabolic pool of platelet adenine nucleotides, loss of the second wave of aggregation induced by ADP, epinephrine and thrombin in samples of citrated platelet-rich plasma, and absence of collagen-induced aggregation. However, in contrast to Maurer *et al*, Hardisty and Mills did not find any characteristic ultrastructural difference between normal platelets and those of the patients.

We have also evaluated a child with albinism and mild hemorrhagic symptoms. Although his case is similar in many respects to the reported cases,[1-6] studies of his defective platelets suggest that he has a previously undescribed variant of the Hermansky-Pudlak syndrome.

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Case Report

The propositus is a 3-year-old Caucasian male. His parents are second cousins, and of Irish descent. Two older siblings of the patient, a girl and a boy, are normal. The pregnancy, labor and delivery were uncomplicated; the albinism was noted shortly after birth. At age 2½, the time of our first observation, his hair was a light blond color, irides were blue, horizontal nystagmus was manifest, and the skin was extremely pale. The findings of the rest of the physical examination were normal.

No petechiae or large hematomas were detected on any of his several visits to the hospital. However, his mother had noted a tendency toward easy bruising after minimal trauma, and bleeding from his gums when deciduous teeth erupted. Injury to a central incisor at age 2 resulted in mild but continuous bleeding for 24 hours. He was circumcised without incident, and had not required subsequent surgical procedures. Blood transfusions had never been necessary. No severe injuries or lacerations had been sustained. The father and mother gave no history of easy bleeding, and could not define any suggestion of a bleeding disorder in other members of the family. The rest of the child's past medical history and family history were noncontributory.

Materials and Methods

Routine laboratory tests and coagulation studies were carried out by standard technics described previously.[7-9] The methods used in this laboratory for obtaining blood, separating citrated platelet-rich plasma (C-PRP), studying the effects of various aggregating agents (collagen, thrombin, adenosine diphosphate [ADP], epinephrine and serotonin) added to C-PRP on the platelet aggregometer, and fixing samples of platelets for examination in the electron microscope were discussed in detail in other publications.[10-12] Similar technics were used in the present investigation. Serotonin determinations on normal platelets and those of the patient were carried out by the method of Takemori *et al.*[13] The mother was carefully instructed to avoid giving the child aspirin and other drugs during the 10 days prior to the laboratory investigations.

Results

General

The patient's hemoglobin, white count, differential, platelet count and urinalysis were normal. Coagulation studies, including prothrombin time, partial thromboplastin time, clot retraction and assays of factors II, VII, VIII, X, and XII were within normal limits. The Ivy bleeding time was 11 minutes on one occasion, and 16 minutes on another (normal time is up to 7 minutes). The availability of collagen-induced platelet factor 3, measured by the decrease in the Russell viper venom time at 5-minute intervals, was abnormal (Table 1). Used in the amount causing a rapid decrease in venom time of normal C-PRP, collagen had a minimal effect on the patient's platelets. Freezing and thawing decreased the venom time in the patient's platelets to the same extent as it did in normal platelets.

Serotonin content of the patient's platelets, measured by the Takemori

Table 1. Collagen-Induced Platelet Factor 3 Availability Measured by Shortening of the Russell Viper Venom Time (VT)

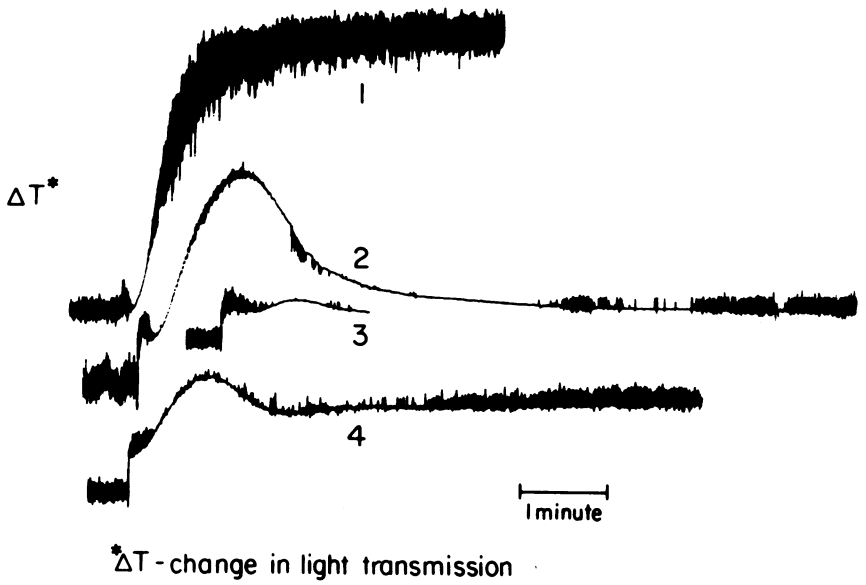
Time (minutes)	VT (seconds)	
	Normal platelets*	Platelets of patient
0	38.5	36.2
5	36.0	36.0
10	33.0	36.0
15	30.0	34.4
20	28.0	33.6
25	27.0	33.0
30	26.0	30.4
Freeze-thaw	9.5	9.4

* Average of 5

method,[13] was 0.059 $\mu g/10^{11}$ cells, about one tenth the concentration found by this technic in normal platelets (0.6–0.8 $\mu g/10^{11}$ cells) in children of similar age. Bone marrow aspirated from the child was examined by light and electron microscopy and revealed no evidence of the pigment-laden macrophages described by other workers in patients with a similar condition. Measurement of urinary glycolipid revealed an elevated excretion of the Gl-1 fraction, details of which will be described in a separate report.[14]

Investigations Using the Aggregometer

Final concentrations of ADP ($1–5 \times 10^{-6}$M), epinephrine (5.5×10^{-6}M), and thrombin (0.1–0.2 units), which produced biphasic responses in samples of normal C-PRP, always resulted in a monophasic or single waves in C-PRP of our patient (Text-fig 1–3). Increased concentrations of various agents produced modified second waves of aggregation. Epinephrine at a concentration of 1×10^{-4}M caused a first phase followed by a gradual increase in light transmission, suggesting slow growth and increase in the number of aggregates (Text-fig 1). Increased amounts of ADP resulted in a greater primary response and slower reversal (Text-fig 2). A final concentration of 1×10^{-4}M ADP produced a single wave, which was not reversible. Thrombin also caused a single, irreversible wave of clumping when added in high concentrations (Text-fig 1), but only a single phase when present in amounts that regularly initiated a massive single wave or a biphasic response in normal C-PRP. The reaction of the patient's C-PRP to collagen revealed a striking difference from the response of normal C-PRP (Text-fig 3). Instead of the massive irreversible single wave, the patient's platelets responded with a slowly



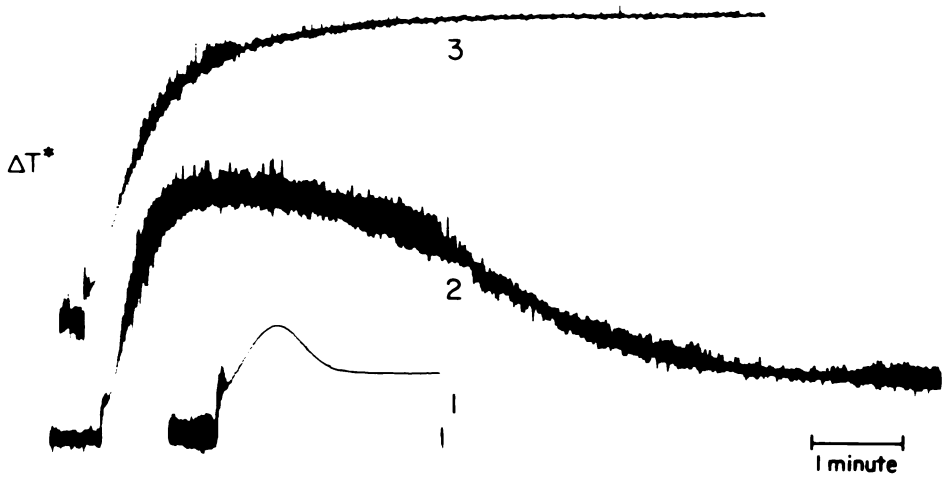
TEXT-FIG 1—Tracings obtained after adding aggregating agents to samples of citrated platelet-rich plasma (C-PRP) from patient. Adding thrombin in a final concentration of 0.1 units/ml of C-PRP and 0.5 units/ml of PRP to the samples recorded in tracings 3 and 2 respectively, resulted in monophasic or single waves of platelet aggregation. Irreversible aggregation was induced by adding a final concentration of 5 units of thrombin/ml of C-PRP to the sample in tracing 1. Epinephrine in a final concentration of 10^{-4}M was added to sample in tracing 4. Monophasic response is followed by slow rise in light transmission, suggesting secondary aggregation. It is possible, however, that aggregates have dispersed and platelets are recovering their discoid shape in this example.

progressive increase in light transmission after the initial phase of platelet-collagen interaction.

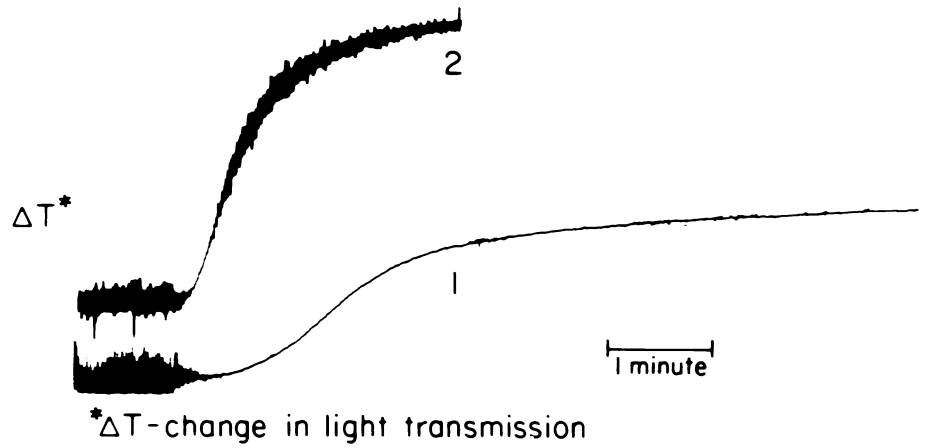
Electron Microscopic Studies

Thin sections of platelets from samples of the patient's C-PRP revealed normal structural features except for decreased numbers of dense bodies. The cells were discoid in shape and had the usual variation in size seen in normal samples (Fig 1). Microtubules, granules and channels of the open canalicular and dense tubular system were present in their usual frequency and location (Fig 2). However, in contrast to normal platelets, which contain an average of 1.4 dense bodies/platelet in thin section, the patient's cells had approximately one dense body in 20 platelets (Fig 1). In addition, the dense bodies present in his platelets were frequently smaller than usual, often resembling irregular clumps rather than spherical, opaque organelles (Fig 2A and 2B).

Examination of platelets on the aggregometer, fixed at intervals during



TEXT-FIG 2—ADP-induced aggregation in samples of patient's C-PRP. Adding ADP to final concentration of 1.5×10^{-6}M caused monophasic response in tracing 1. In final amount of 1×10^{-5}M. ADP also produced single wave of aggregation recorded in second tracing. At final concentration of 1×10^{-4}M. agent caused irreversible aggregation. demonstrated in tracing 3.



TEXT-FIG 3—Response of patient's C-PRP to addition of collagen in tracing 1 is compared to the reaction of a normal sample of C-PRP to same agent shown in tracing 2. Collagen induced early changes in patients as in normal cells. indicated by narrowing of baseline. However. instead of rapid increase in light transmission followed by irreversible aggregation evident in tracing of the normal sample. patient's C-PRP developed modified pattern of response. Light transmission increased steadily for first 3 minutes. then slowed to gradually ascending phase. which did not reach a maximal decrease in optical density during the period of recording. Pattern evident in patient's tracing suggests inadequate availability of secretory products essential for rapid development of irreversible aggregation.

the transformation induced by aggregating agents, revealed alterations consistent with normal platelet function. Platelet samples exposed to amounts of epinephrine, ADP and thrombin that produced single waves of clumping caused the same changes observed previously in normal platelets pretreated with aspirin, quanadinosuccinic acid or atropine.[15,16] Initial change in shape and minor internal transformation were noted in loose platelet aggregates, but granule fusion, formation of tight mosaics and massive aggregation did not occur (Fig 2C). Disaggregation and recovery of unaltered platelet appearance occurred 5–15 minutes after the exogenous agent was added (Fig 2D). Higher concentrations of ADP and thrombin produced irreversible aggregation, and caused alterations in the form and internal organization of platelets that were consistent with viscous metamorphosis.

Collagen-induced aggregates were of particular interest. Samples obtained at intervals during the slow increase in light transmission on the platelet aggregometer contained smaller aggregates than were found in collagen-clumped, normal C-PRP fixed at similar times, but the changes in individual cells were identical (Fig 3). Granules were centrally clumped, surrounded by microtubules and microfilaments and often fused. Pseudopods were intimately entwined, forming tight mosaic patterns (Fig 4). The changes were consistent with the complete transformation induced in normal platelets by collagen.

Discussion

The findings of biochemical, physiologic and ultrastructural defects in the platelets and the normal values of coagulation tests suggest that our albino patient's mild bleeding disorder is due to platelet dysfunction. A vascular disorder, similar to the pseudohemophilia described by Hermansky and Pudlak [1,2] in their patients with albinism and widespread deposits of ceroid-like pigment in endothelium, was not ruled out in our patient. The absence of pigment-laden macrophages in aspirates of bone marrow, however, may indicate that the child's vascular endothelium is similarly devoid of this material. His youth alone may account for the absence of storage pigment, since he does have an increased excretion of glycolipid into the urine.[14]

The patients studied by Maurer *et al* [4] were found to have pigment inclusions in the bone marrow, and cases evaluated by Mills and Hardisty may also have had this finding. Yet, the bleeding disorder in these recently studied cases has been related to abnormal platelet function rather than to a vascular defect.[3–5] The sensitive technics used in newer investigations were not available when Hermansky and Pudlak found normal platelet

activity in their patients.[1,2] Evaluation of their cases by current methods would probably reveal platelet defects similar to those found recently. Ceroid deposits may affect the integrity of blood vessels, as was suggested.[1,2] However, the rarity of a hemorrhagic disorder occurring simultaneously with albinism and storage of ceroid pigment renders it unlikely that bleeding is due to pseudohemophilia in some cases and to platelet abnormalities in others. We suggest that defective platelets in patients with this triad are the basic cause of hemorrhagic symptoms, and that the term *pseudohemophilia* in reference to the bleeding disorder should be discarded.

Three basic mechanisms are involved in the hemostatic function of blood platelets: adhesion (aggregation), contraction and secretion.[17,18] Complete clot retraction of blood samples from our patient, and the development of tightly molded mosaics of platelets, with complete internal transformation in collagen-induced aggregates, indicated that the contractile capacity of his cells was normal. The normal, primary phase of clumping, measured by aggregometry after the addition of epinephrine, ADP, collagen and thrombin to samples of his platelet-rich plasma, indicated that his platelets were capable of normal adhesion and aggregation. The low levels of platelet serotonin, decreased numbers of dense bodies, poor availability of platelet factor 3 and abnormal secondary aggregation are all compatible with a defect in the secretory capacity of his platelets. The abnormality is not as severe as that in other patients with albinism, whose platelets did not aggregate with collagen, failed to develop second waves of epinephrine- and ADP-induced aggregation and were almost completely depleted of serotonin and the nonmetabolic pool of adenine nucleotides.[4,5] Our patient, therefore, appears to be a variant of the Hermansky-Pudlak syndrome, though the marked abnormalities noted in other patients may develop when he is older.

The results of this study support the concept that dense bodies are related closely to secretory function, and that a decreased number or absence of electron-opaque organelles is reflected in a characteristic pattern of platelet dysfunction.[19,20] Maurer *et al* reported a complete absence of dense bodies in platelets from patients in whom the platelet secretory function was absent.[4] We found a reduced number of dense bodies in our patient's platelets and a diminished but still demonstrable capacity to secrete. Mills and Hardisty, however, observed no characteristic difference in the ultrastructure of platelets from their patients when compared to cells obtained from normal donors.[5] This apparent disagreement with the findings of Maurer *et al* and ourselves is perplexing. The relationship between the number of dense bodies in mammalian platelets and their

content of serotonin has been clearly established.[21,22] Platelets from the 2 patients studied by Mills and Hardisty contained from 2–6% of the level of serotonin found in normal cells.[5] It is extremely doubtful that platelets containing such minute amounts of serotonin could have a normal complement of dense bodies, since their formation in platelets depends on the concentration of the amine. Problems involved in fixation of dense bodies for study in the electron microscope have led other workers to consider them to be rare constituents of human platelets,[21,22] and similar difficulties may have affected the observations of Mills and Hardisty.[23,24]

Another important feature of normal platelet physiology was revealed in this study of platelets with diminished secretory capacity. An amount of ADP considerably greater than that necessary to stimulate the release reaction and irreversible secondary aggregation of normal platelets was required to cause secretion of sufficient endogenous chemical substances from the patient's platelets to sustain even a modified second wave. This finding might suggest that the patient's platelets are somewhat resistant to the influence of ADP. Yet examination of his platelets from the first wave indicated that his cells underwent the same internal and external changes as normal platelets.[12] A more reasonable explanation for the increased amount of ADP required to induce secondary aggregation is that a larger number of the patient's platelets must be stimulated to extrude endogenous products in order to obtain a concentration sufficient to cause secondary aggregation. Thus, the triggering of platelet contraction necessary for secretion of endogenous chemical constituents does not develop in an all-or-none fashion, but in a manner proportional to the stimulus applied. Either some platelets in samples of platelet-rich plasma are relatively refractory to the lower concentration of ADP, or else the intensity of contraction in individual cells reflects the concentration of the exogenous agent. An increasingly forceful contraction of more platelets under the influence of a greater concentration of ADP would release diminished but adequate amounts of endogenous platelet ADP to initiate the second wave of aggregation. The reaction of platelets revealed by these experiments is strikingly similar to the response of muscle to increasing work loads and secretory cells of endocrine organs to graduated levels of stimulation.[25,26]

The nature of the modified second wave evident in tracings of ADP- and collagen-induced biphasic responses of the patient's platelets are also of interest. Despite secretion of sufficient endogenous substance to initiate the second wave, the self-sustaining process proceeded slowly. A similar retardation in development of secondary aggregation was ob-

served in previous experiments with normal PRP when the release reaction was partially blocked by atropine.[15] The release reaction is not only necessary to initiate secondary aggregation, but must also be sustained at a rate in excess of that possible in our patient's cells in order for rapid growth of large platelet aggregates to take place. These findings indicate the dependence of secretion on platelet contraction, and the essential role of secretion in the rapid formation of large platelet aggregates.

The relationships between the albinism, ceroid storage and absence of platelet dense bodies in previously reported studies and the albinism, increased excretion of glycolipid and decreased number of platelet dense bodies in our case are unknown. A defect in melanogenesis might be related to an inability to form dense bodies. Both organelles develop from preformed structures of the cells (pre-melanosomes[27] and granules[23]), and after formation both are inherently electron opaque. However, the albinism in our patient was not due to defective melanogenesis *per se*, but to a marked decrease in melanocytes.[14] Melanosomes observed in the rare melanocytes present in his hair bulbs were pigmented. The nature of the albinism in other cases of Hermansky-Pudlak syndrome has not been described. If the lack of skin pigmentation and light hair color in these cases are also due to a lack of melanocytes rather than a defect in melanogenesis, then this disorder represents a distinct form of albinism.[27,28] Thus, the ceroid storage and decreased number of dense bodies in platelets are not necessarily related to classic albinism, but to a type of hypopigmentation unique to patients with the Hermansky-Pudlak triad. Individuals with classic albinism have a normal complement of dense bodies in platelets, normal platelet function and a normal number of melanocytes with unpigmented melanosomes. Clarification of the unique form of albinism in Hermansky-Pudlak syndrome may help to elucidate the basis for the storage disorder and the defect in the formation of platelet dense bodies.

Summary

Platelets from a child with albinism, increased urinary excretion of glycolipid and a mild hemorrhagic disorder were chemically, physiologically and morphologically abnormal. The defects in platelets included a reduced concentration of serotonin, a decreased number of dense bodies and a diminished release reaction essential for initiating and propagating the second wave of irreversible platelet aggregation. The patient appears to have a variant of the syndrome, initially described by Hermansky and Pudlak, of albinism, ceroid-like pigment storage in bone marrow, and easy bleeding.

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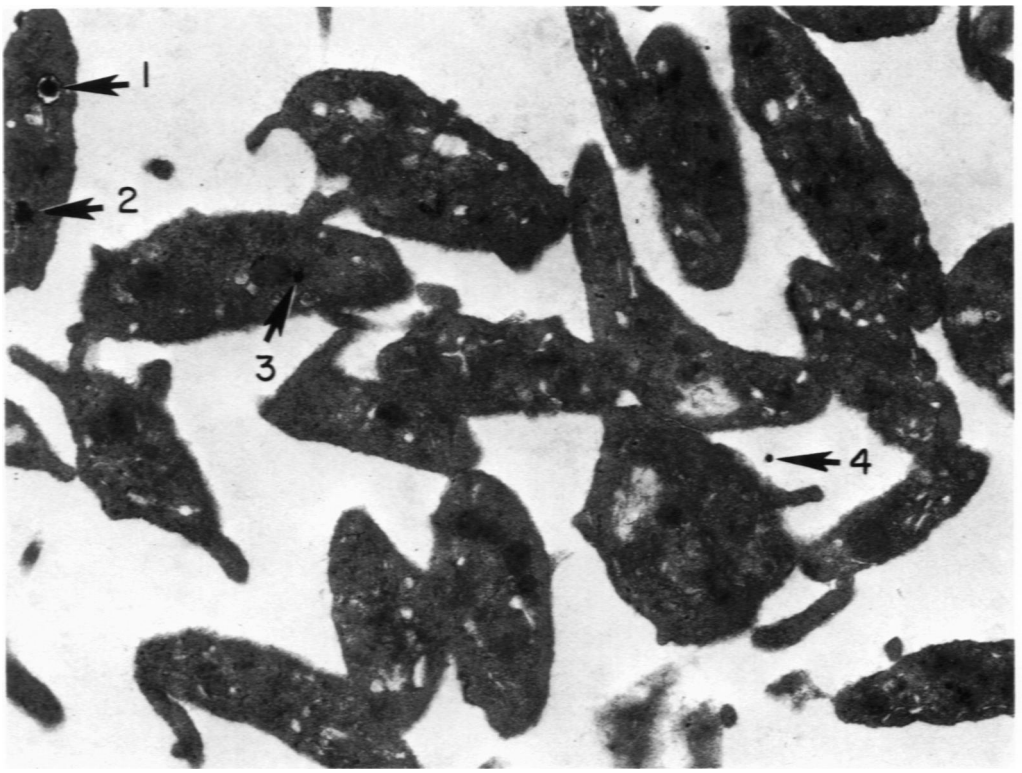
Fig 1—Sample of C-PRP from patient, prepared for study in electron microscope. Single platelet contains two dense bodies (*1* and *2*). Particles at *3* and *4* are stain aggregates not to be confused with dense bodies of platelets. Normal cells contain approximately 1.4 dense bodies/platelet. The patient's cells revealed about one dense body/20 platelets in thin section (× 21,500).

Fig 2—Cells in **A** and **B** reveal discoid shape, granules (*G*), microtubules (*MT*), mitochondria (*M*), glycogen (*Gly*) and canalicular system (*CS*) characteristic of normal platelets. Dense aggregates indicated by *1* and *2* in **A** were seen in the patient's cells containing dense bodies. They have opacity of platelet dense bodies, but are smaller and more irregular in shape. The organelle (*1*) in **B** also resembles dense body, but its opacity is similar to that of nucleoids in granules indicated at *2* and *3*. Platelet in **C** is from aggregate induced by final concentration of 2×10^{-6} M ADP and fixed during first wave. Clumping of granules in cell center and enclosure by band of microtubules are typical of internal changes observed in normal platelets. Platelets in **D** are from sample fixed during reversal of second wave of aggregation induced in patient's C-PRP by 10^{-5} M ADP (A, × 25,000; B, × 26,500; C, × 26,800; D, × 19,300).



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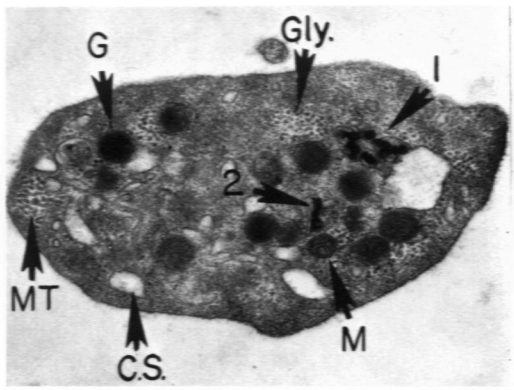

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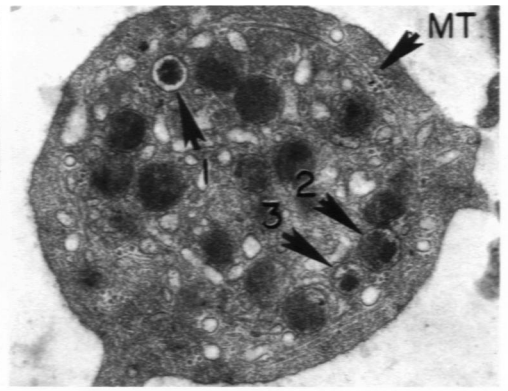

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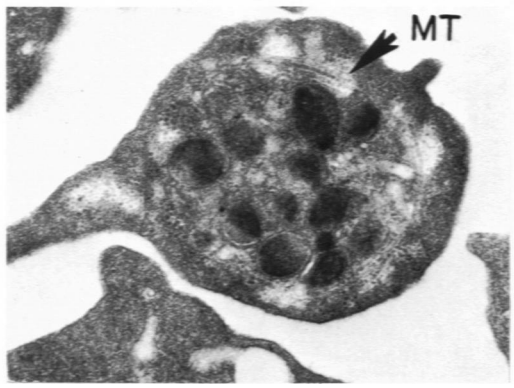

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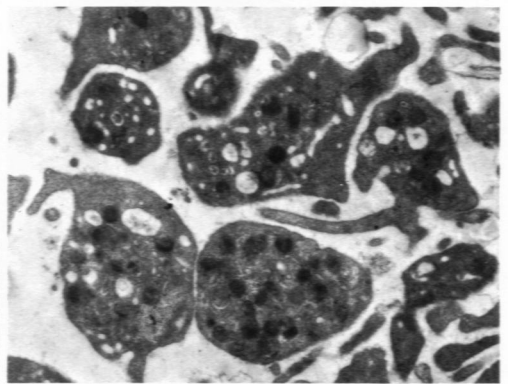

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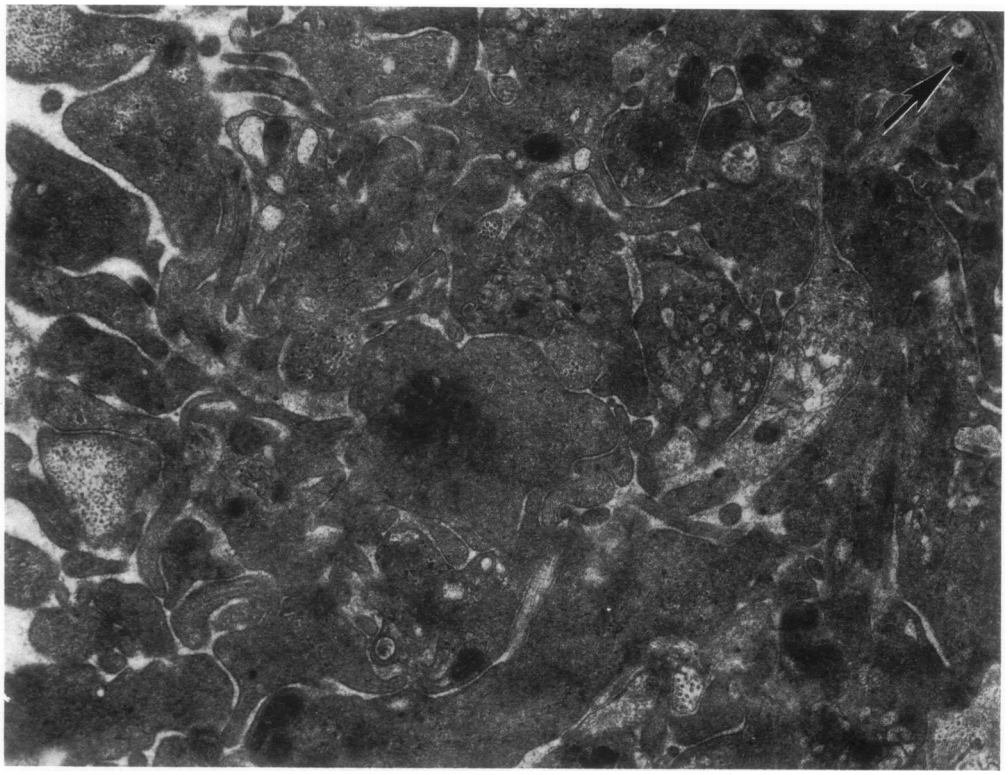

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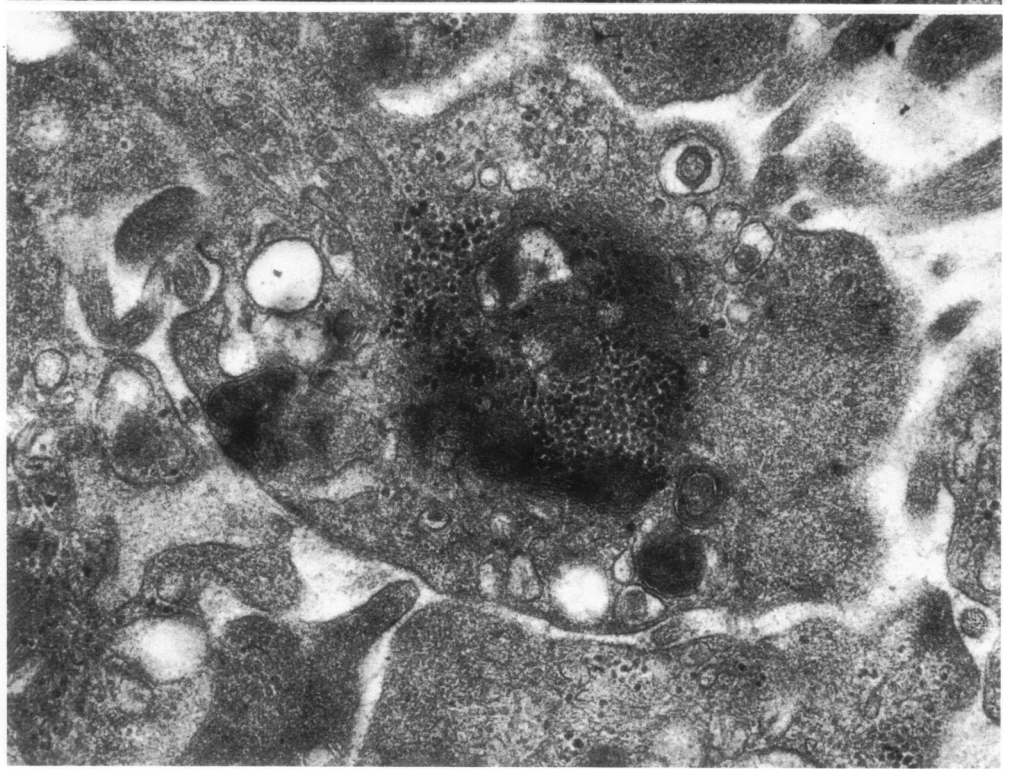

Fig 3—Collagen-induced platelet aggregation. Aggregates that developed in patient's C-PRP were less numerous and smaller than those found in normal C-PRP. Appearance of aggregates was indistinguishable from normal, however. Cells and pseudopods were molded into tight mosaic consistent with complete viscous metamorphosis. Dense body is present in cell mass (*arrow*) (× 17,100). **Fig 4**—Platelet from patient's C-PRP after aggregation by collagen. Central accumulation and fusion of most organelles in cell center is characteristic step in normal platelet viscous metamorphosis (× 31,500).