

The Blood Platelet: Electron Microscopic Studies

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I. Introduction

The last formed elements of the blood to be discovered were the blood platelets. Described for the first time by Donné in 1842 under the name of *globulins* they were further described in 1847 by Zimmerman who noticed their tendency to form aggregates. Some years later the German anatomist Max Schultze (1865) gave a clear description of the platelets but he interpreted them as being destroyed erythrocytes.

During this initial period two other authors who were interested in the study of these elements were Vulpian (1873) and Osler (1874). Vulpian pointed out their tendency to adhere to surfaces, and admitted the existence of a relation between these elements and the fibrin formation during clotting. Osler described the platelets and called them the *third element of the blood*. In his point of view they are independent from the other elements of the blood.

The platelets have taken on more importance as a result of the work of Hayem (1877) and Bizzozero (1882). For that reason their discovery is frequently at-

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tributed to these authors. Hayem (1877), who identified the platelets in the blood of a great number of vertebrates, named them *hematoblasts*, because he thought they were the precursors of the erythrocytes, and admitted that they are accelerating agents of the blood coagulation. Bizzozero (1882) observed them for the first time in the blood of a living animal, and in his opinion they were independent from the other blood cells. He also noticed their intervention in clotting and in plug formation.

After an early period, when studies on the platelets were concerned primarily with the question of their independence of the other blood elements, the studies in this area have taken progressively a physiological and pathological direction. Their morphological description became more accurate, and their importance in the coagulation phenomenon clearer. In this connection the introduction of new techniques which permitted their easier isolation was of great importance.

The small dimensions of the platelets have always limited their morphological study but with the development of the electron microscope new possibilities were opened for their examination. Their small dimensions, a limitation for their observation under the optical microscope, made them proper candidates for study with the electron microscope. They are also suitable material for examination with the electron microscope because of their ability to spread out when placed on a hydrophilic surface. In these conditions the platelets become almost completely transparent to the electron beam. For all these reasons, in a period when the application of the electron microscope to biological studies was still primitive, the platelets were one of the few biological materials on which observation was possible.

The first description of these elements with the electron microscope was published by Wolpers and Ruska in 1939 (1939a). This work, carried out in the same laboratory where Van Borries and Ruska developed the electron microscope, is one of the first instances in which this apparatus was employed in a biological study.

After a hiatus, in part corresponding to World War II, several articles were published which contained observations on the platelets with the electron microscope. The method almost generally employed was the spreading technique, with or without shadowing.

With the advent of the ultramicrotomy techniques a new chapter was opened in the area of submicroscopic research. The application of these techniques to the study of the platelets has given a new image of these elements and has added new perspectives to their study.

This article consists primarily of a review of the contributions of electron microscopy to the study of the morphology, physiology, and pathology of the blood platelets.

II. Submicroscopic Morphology of Normal Blood Platelets

A. PRELIMINARY OBSERVATIONS WITH THE ELECTRON MICROSCOPE

Before the introduction of ultrathin sections the platelets had been observed in the electron microscope with the following techniques:

- a. Directly after isolation and fixation (Wolpers and Ruska, 1939a, b; Bessis, 1948; Braunsteiner, 1949).
- b. After spreading, with or without shadowing (Bessis, 1948; Bessis and Bricka, 1949; Rebusk, 1949).
- c. After mechanical destruction or chemical digestion (Bessis, 1950).
- d. With the replication technique, with or without shadowing (Bessis, 1953; Bricka and Tabuis, 1953).



FIG. 1. Diagrammatic representation of the spontaneous spreading of a platelet on glass as seen with phase contrast microscope. (Courtesy of Dr. M. Bessis.)

Of all these methods, it was the spreading technique that gave the best results. This technique is based on the property of some elements to spread out when placed on a solid surface (Fig. 1). When this surface is a silicone or Formvar membrane, the spreading is intensified and the thickness of the elements considerably reduced. They are transformed to a thin layer of protoplasm transparent to the electron beam.

Bessis and Burstein (1948), who studied this phenomenon with the ultra-microscope, also employed the spreading technique in the study of the platelets with the electron microscope.

When the platelets are fixed immediately after collection they have a round or discoidal shape (*circulatory form*). Fixed some minutes later, several thin and long protoplasmic processes appeared (*dendritic form*). As the time between collection and fixation increases, these pseudopods by progressive enlargement are transformed into thin sheets (*transitional form*), which coalesce and form a thin veil of hyaloplasm (*spread form*) (Figs. 2, 3).

The platelets have a very great spreading capability. Placed in a hydrophilic surface they spread out and after some minutes they have ten times their normal surface. Under these conditions they are almost completely transparent to the electron beam.

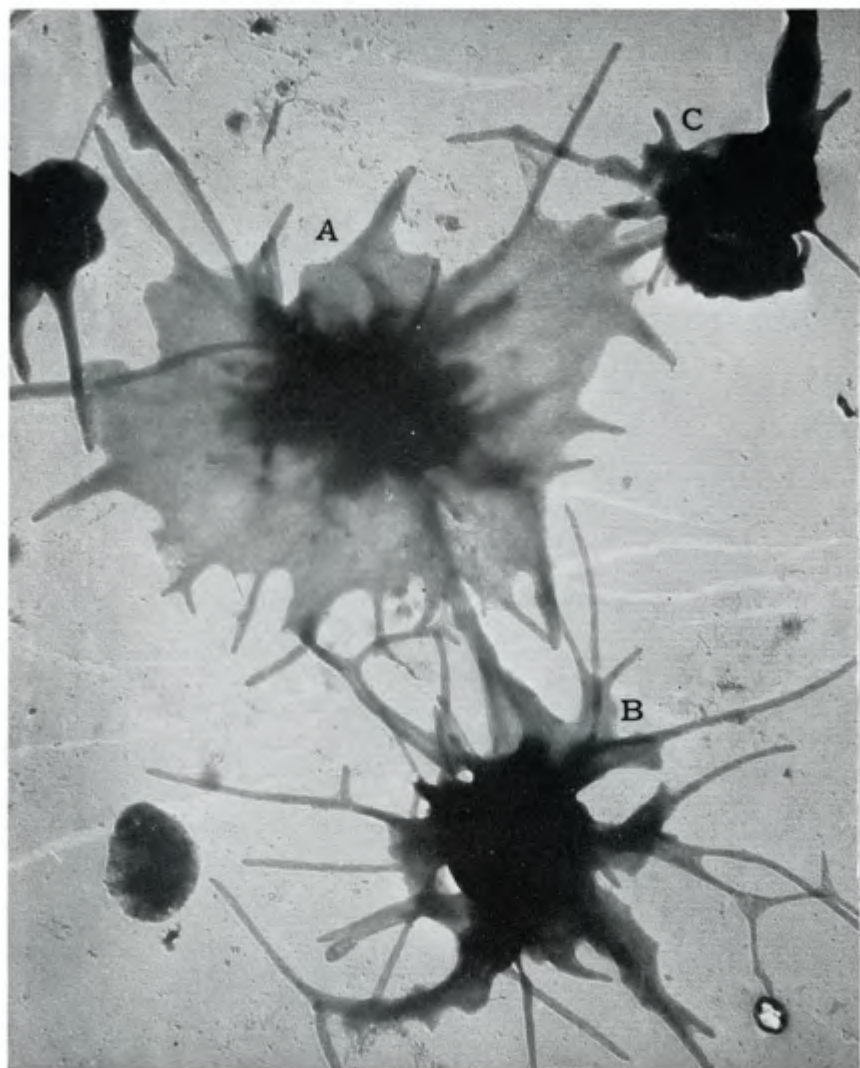


FIG. 2. Human blood platelets. Fixation in buffered osmic acid; shadowed with Pt-Pd. A, normal spreading form with a wide zone of hyaloplasm; B, transitional form where the dense chromomere is prominent but the hyalomere is reduced to a number of pseudopods; C, distinct chromomere but no hyalomere. $\times 8650$. (Courtesy of Dr. G. Bloom.)

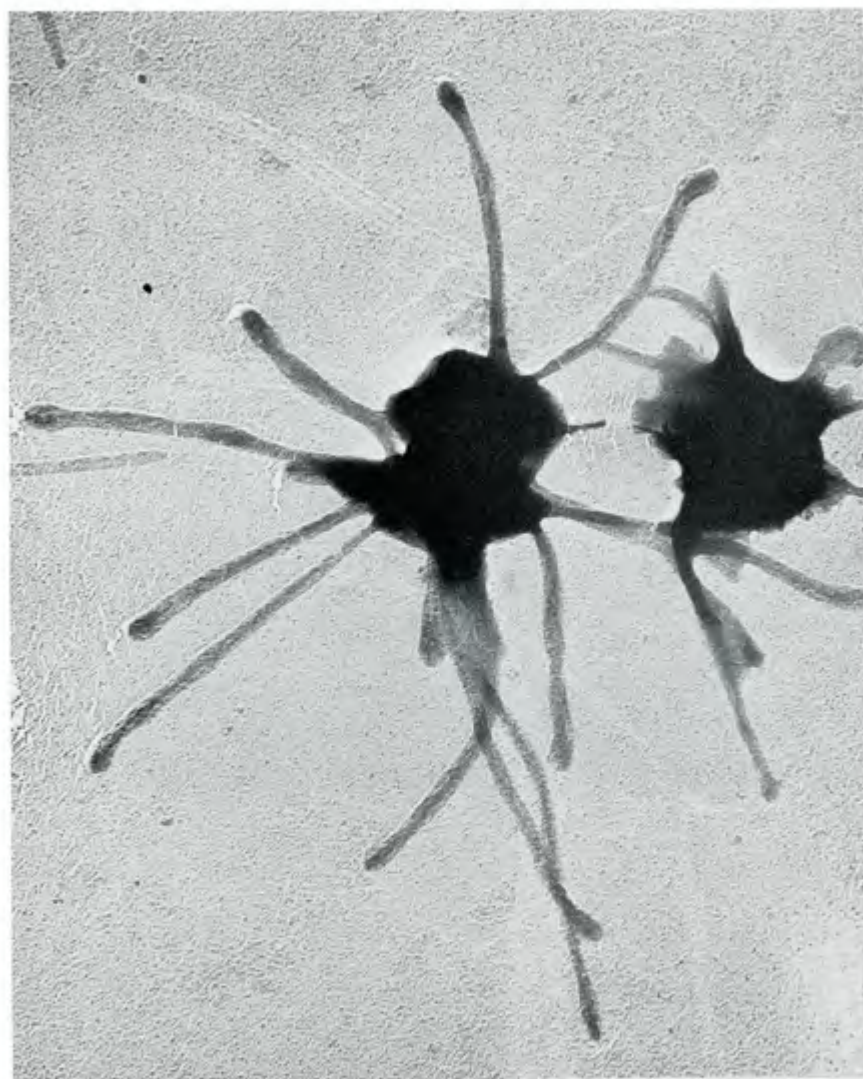


FIG. 3. Human blood platelets. Fixation in buffered osmic acid; shadowed with Pt-Pd. Dendritic forms. $\times 12,200$. (Courtesy of Dr. G. Bloom.)

Under the optical microscope, in a stained film of peripheral blood, the platelets consist of a ground substance, homogeneous, colorless, or slightly basophilic (*hyalomere*), in which are scattered or are centrally grouped granules of violet color (*granulomere* or *chromomere*). With the electron microscope, after spreading, two zones are also observed in the platelets: one central, electron-opaque with several dense granules ($0.2\text{--}0.3\ \mu$ in diameter), and another peripheral and thinner, corresponding to the hyalomere (Fig. 4).

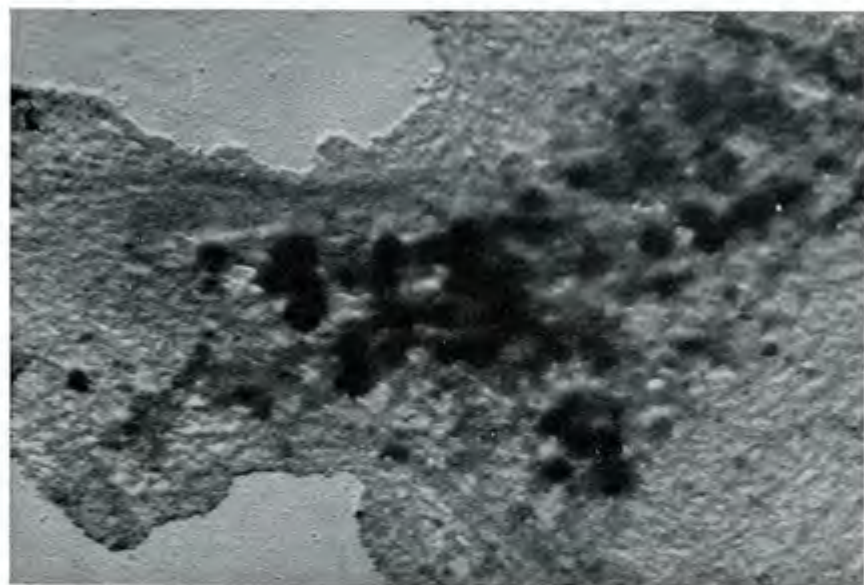


FIG. 4. Human blood platelet. Fixation in buffered osmic acid; shadowed with Pt-Pd. Chromomere of a platelet, thin enough to be penetrated by the electron beam, showing a number of granules of somewhat varying sizes. $\times 16,250$. (Courtesy of Dr. G. Bloom.)

The study of the hyalomere ultrastructure has been the principal subject of the numerous studies reported in this first period. In agreement with Bessis (1948) and Braunsteiner (1950) the hyalomeres are granulofibrillar. Bessis and Bricka (1948) indicated that their aspect depends on the fixative employed: hyaline with osmic acid, alveolar with formol, and fibrillar with alcohol. The fibrils are formed by small granules of about 50 to $75\text{ m}\mu$, and they are radiating or circular. Haguénau and Bernhard (1952), who utilized spreading of platelets in a study of fixatives, observed that the ground substance is fibrillar after alcohol and chromic acid and homogeneous when osmic acid or formol are employed. The fibrils observed in the platelet hyalomere were interpreted some years later as artifacts of fixation (Bessis, 1954; Bloom, 1955).

Several other observations were made in this first period about the morphology of the platelets, particularly in relation to the modifications of their shape in different experimental and pathological conditions. They are referred to in other parts of this article.

We can conclude that before the introduction of ultrathin sections the progress achieved in our knowledge about the platelet has been small. However these studies are important from the technical point of view. The platelets have been good material for the study of the fixing agents, and the analysis at the present time of the early work is a good example of the technical evolution in the study of a biological material under the electron microscope.

B. THE ULTRASTRUCTURE OF THE NORMAL BLOOD PLATELET

1. General Description

The first observations of thin sections of blood platelets were reported in 1955 by Bernhard and Lepus (1955), DeMarsh *et al.* (1955), and Rinehart (1955). After these articles the publications on this subject are numerous (see Marcovici and Gautier, 1959; Marcovici *et al.*, 1961). In agreement with these studies the platelets are round or oval elements about 2 to 5 μ in diameter, limited by a membrane with an irregular outline. Inside we can distinguish a finely granular ground substance (hyalomere) and different types of granules and vacuoles (granulomere) (Figs. 5, 6). The aspect of the platelets observed inside capillaries of different organs (bone marrow, spleen, kidney, or lung) is identical but generally they are longer and their outline is sometimes more irregular.

In the thin sections of platelets isolated by centrifugation, there are frequently observed between the platelets with the aspect previously described, others, clear as though they have lost their ground substance. In these elements the outline of the membrane is very regular and their granules are denser (Figs. 4, 7).

2. Membrane

The existence of a limiting membrane in the platelets, admitted by Bizzozero, has been questioned for a long period. Yet in 1954 in a book devoted to the study of the platelets, Maupin described this observation, inspired by Tullis: "Parmi les acquisitions de la morphologie pure, nous retiendrons: l'absence de forme propre, ou plus exactement la grande plasticité des plaquettes qui semble indiquer qu'elles sont démunies de membrane."

Examined under the electron microscope, platelets show a distinct plasma membrane (Figs. 5 to 7). The constitution of this membrane is evident in preparations fixed with potassium permanganate where it is easy to recognize

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FIG. 5. Normal human platelets. Fixation in osmic acid; embedded in methacrylate; stained with uranyl acetate. Platelets are limited by a membrane with irregular outline. In the hyalomere are dispersed the granules and vacuoles which form the granulomere. Two clear platelets are indicated by arrows. $\times 6500$.

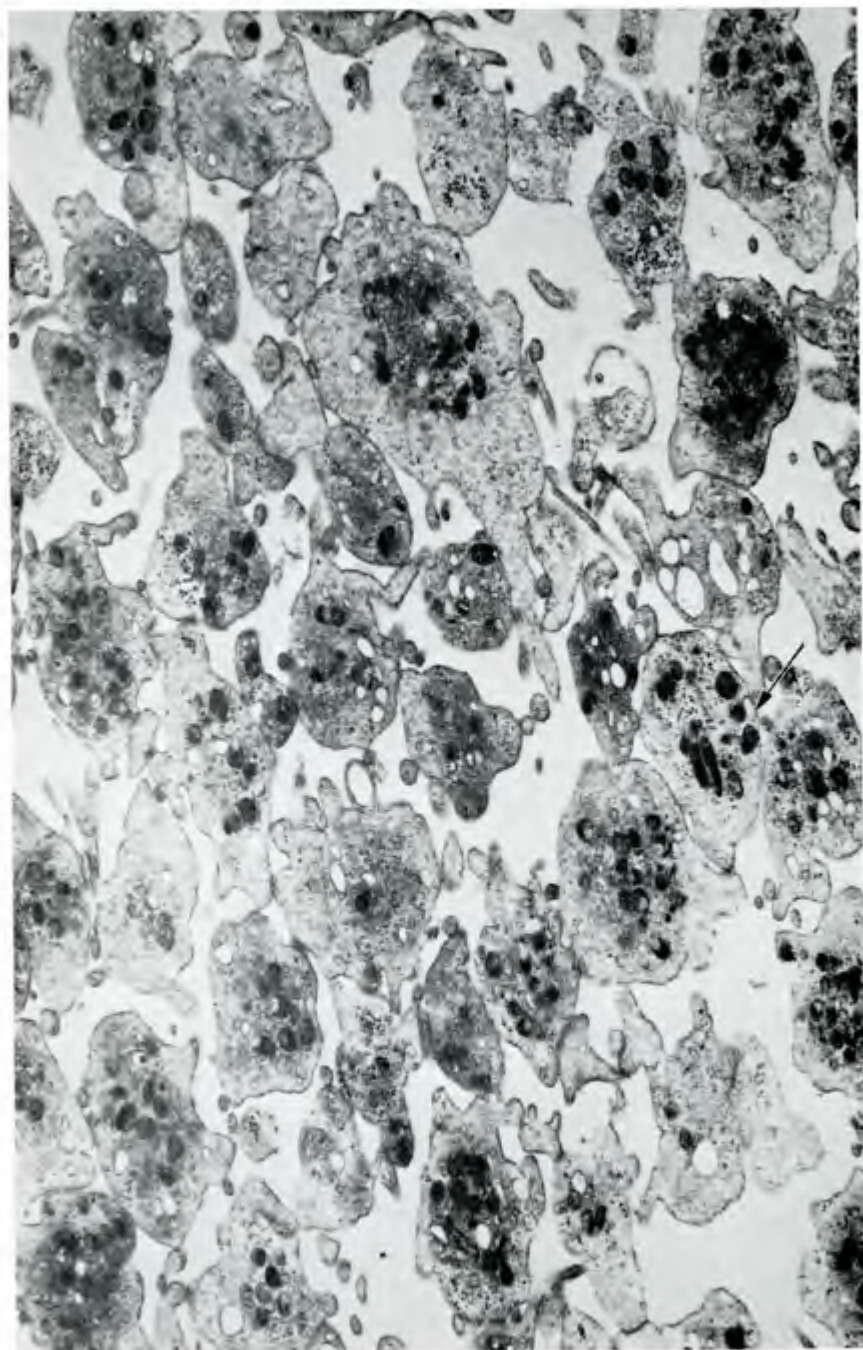


FIG. 6. Normal human platelets. Same technique as Fig. 5. The different granulomere components are seen. Arrows indicate a platelet with drum-stick granules. $\times 11,000$.



FIG. 7. Human platelet, *clear form*. The hyalomere has lost its contrast. Some vesicles and granules are denser than usually seen. $\times 44,000$.

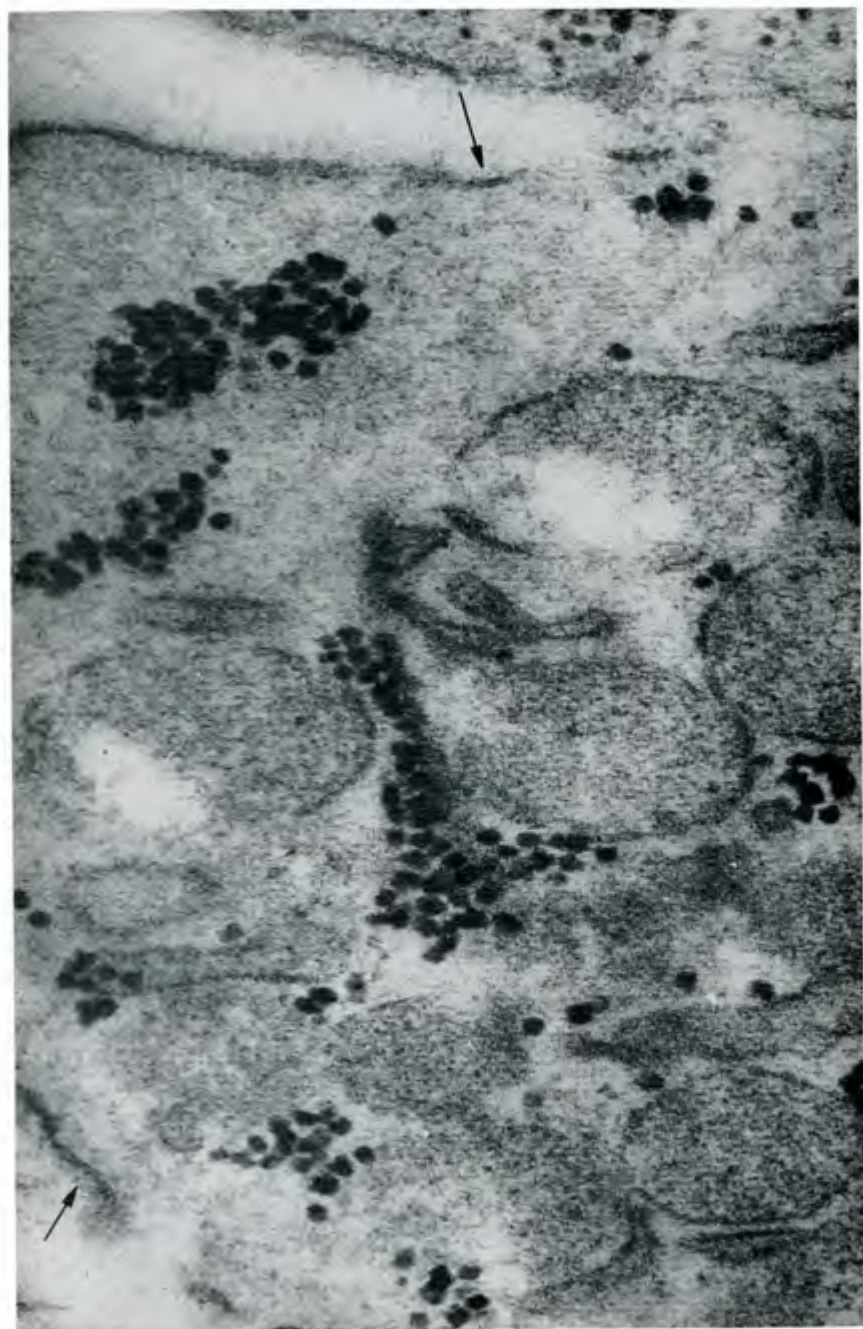


FIG. 8. Normal human platelet. Fixation in potassium permanganate; embedded in Araldite; stained with uranyl acetate. Arrows indicate points on the platelet membrane where their elements (unit membrane) are evident. Between the dense granules with recognizable membrane are seen aggregates of the glycogen granules. $\times 126,000$.

the unit membrane of Robertson (1959; David-Ferreira, 1961b). The platelet membrane is about 78 Å thick and is formed by three layers: two dense layers of 20 Å each separated by an intermediate brighter one (Fig. 8). This membrane, which is morphologically identical to the cellular membranes, also has some identical physiological properties.

3. Hyalomere

The ground substance of the blood platelets is homogeneous or finely granular. It can be more or less dense and sometimes completely transparent (*clear platelets*) (Fig. 7). Variations in the hyalomere density have been described by Feissly *et al.* (1959) in a study of the modifications of the platelets in stored blood. In agreement with these authors the number of clear platelets increases with the length of time of blood storage. Probably the clear platelets observed in sections of peripheral blood are degenerated elements.

In the platelet hyalomere the ribosomes are absent or very scarce.

4. Granulomere

The granulomere or chromomere is the aggregate of granules and vesicles dispersed in the hyalomere.

Bernhard and Lepus (1955) recognized in the granulomere the following elements: mitochondria, vesicles filled with a homogeneous substance, and clear vesicles.

According to Schulz and associates (1958) the granulomere components are divided into four types: α , β , γ , δ .²

a. Dense Granules (Specific Granules; Azurophilic Granules; α -Granulomere). The dense or specific granules are the most abundant elements of the granulomere. In agreement with several authors (Rinehart, 1955; Sueyasu and Tageshige, 1956; Goodman *et al.*, 1957) they correspond to the azurophilic granules seen with the optical microscope. They have an oval shape and are about 120 to 300 m μ long. They are dense (*granulations denses* of Feissly *et al.*, 1960) with a finely granular matrix which is denser after staining with uranyl acetate. They are surrounded by a membrane formed by three layers (Fig. 8). Sometimes inside the dense granules denser zones are observed, generally excentric, and occasionally small lobes are seen on their surfaces (Fig. 21).

According to some authors (Rinehart, 1955; Bernhard and Lepus, 1955; Sueyasu and Tageshige, 1956; Watanabe, 1956, 1957) the dense granules derive from the platelet mitochondria. According to others (Jones, 1960) they are formed in the megakaryocytes.

² Feissly *et al.* (1960) classified them in two groups: the dense granules and the clear elements.

b. Mitochondria (β -Granulomere). The platelet mitochondria described under the optical microscope by Cowdry (1914) are clearly demonstrated with the electron microscope. They are few (one or two per section of platelet), small (0.15–0.20 μ), and have few cristae (2 or 3 per mitochondria).

c. Clear Elements (Vacuoles, Vesicles, and Tubules; γ -Granulomere). Feissly and associates (1960) called clear elements (*éléments clairs*) the aggregate of microvesicles, tubules, and vacuoles observed in the platelets (Fig. 6).

Several hypotheses have been developed concerning their origin and significance. Some authors believe the vesicles and vacuoles originate from the Golgi apparatus of the megakaryocytes, others that they are elements of the endoplasmic reticulum or are still vesicles and vacuoles of pinocytosis. We have arrived at the conclusion that some of the tubules and vacuoles result from phenomena of active incorporation (David-Ferreira, 1961a, b), but we cannot exclude the possibility that some of the clear elements originate in the Golgi apparatus of the megakaryocytes.

According to Jean and Racine (1962) the clear elements do not represent a homogeneous group of organelles. Combining cytochemical methods with electron microscopy they have divided these elements in two groups: one formed by the PTA-positive elements that can correspond to pinocytosis or phagocytosis vesicles and another group PTA-negative, probably derived from the megakaryocyte Golgi apparatus or endoplasmic reticulum.

d. Syderosomes (δ -Granulomere). Among the granulomere elements of the platelets some corpuscles similar to syderosomes have been described (Schulz *et al.*, 1958). Their interior is clear but in their margins dense granules similar to ferritin are seen with a diameter of 55A.

Policard and associates (1959) in a study on rat platelets never observed this kind of granule. Neither have we in preparations of human blood platelets isolated *in vitro* and in rabbit platelets observed inside the pulmonary blood capillaries (David-Ferreira, 1962). More recently Haguénau and associates (1963) identified them in human platelets (Figs. 9, 10). The δ -granules described by Schulz and associates (1958) are very similar to the corpuscles described by Giesecking (1958) in the alveolar cells of the lung after injection of iron hydroxide. This fact and the ability of the platelets to pick up substances from the milieu are the basis of the hypothesis that the δ -granules are a result of an active incorporation by the platelets (David-Ferreira, 1962).

e. Other Granulomere Components. Besides the granulomere elements previously described the following other types of granules have been described in the platelets:

i. The "drum-stick granules" described in the thrombopathia of Willebrand-Jürgens as characteristic of that disease (Schultz *et al.*, 1958).

ii. Granules about 180 to 200 A. in diameter observed in normal human platelets after staining.

iii. Dense granules about 120 A. described under the name of "liberalisierte α -substanz" (Schulz and Hiepler, 1959).

The granules of groups ii and iii have been named the ε -granulomere (David-Ferreira, 1962).

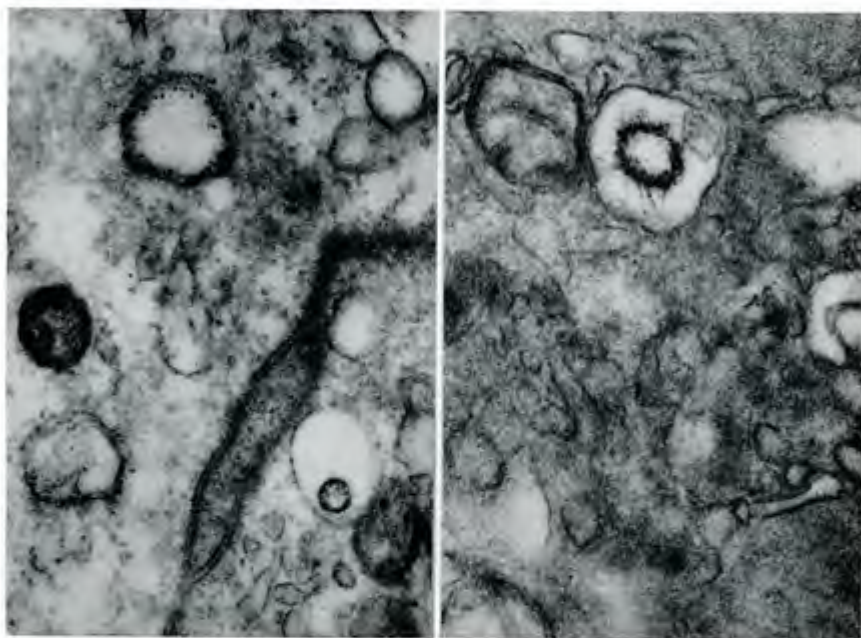


FIG. 9. Human blood platelet. Between dense granules and vesicles a syderosome is seen. $\times 42,000$. (Courtesy of Dr. F. Haguenau.)

FIG. 10. Human blood platelet. Syderosome. $\times 82,000$. (Courtesy of Dr. F. Haguenau.)

i. *The drum-stick granules.* In blood platelets of patients with the thrombopathia of Willebrand-Jürgens a special type of granule has been described by Schulz *et al.* (1958). In reference to their shape these authors called them the drum-stick granules ("trommelschlegelgranula").

These granules are 1 μ long and 500–2000 A. in width. They are surrounded by a membrane, and their matrix has the same aspect and staining properties as the α -granules (Jean *et al.*, 1963b). Schulz and associates (1958) reported these granules in five cases of the Willebrand-Jürgens thrombopathia. In agreement with these authors the number of the drum-stick granules per platelet is highly variable. In one case they observed several of these granules in each platelet; in another case there were but a few. According to Schulz *et al.* (1958) the drum-

stick granules are characteristic of the thrombopathia of Willebrand-Jürgens and are never observed in normal platelets. They advanced the hypothesis that these granules are a morphological expression of the genetic alteration of that disease.

The drum-stick granules have also been described by Jean (1961) in one case of Willebrand-Jürgens, four cases of thromboasthenia and one case of reticulo-endotheliosis. They have also been described by Dalton and associates (1961) in the platelets of leukemic mice.

In agreement with Jean (1961) it is not possible to consider them as characteristic of Willebrand-Jürgens thrombopathia.

Also, the observation of the drum-stick granules in normal human platelets is not very rare (Figs. 5 and 11). They are elongated, and one or both of the extremities are dilated or have a lancet form. They are surrounded by a membrane and their contents are identical to the dense granules. In a previous study (David-Ferreira, 1961b) it was concluded that these granules are not exclusively characteristic of the platelets of patients with the thrombopathia of Willebrand-Jürgens. More recently Jean *et al.* (1963a) in a study of the abnormalities of the dense granules concluded that the drum-stick granules are more frequent in platelets subjected to several experimental conditions, e.g., aging *in vitro*, incubation with diastase, and in some platelet disorders.

ii. *Glycogen granules; ribosomes (ϵ -granulomere)*. Very often dense particles with a diameter lower than 200 A. are observed in the platelet hyalomere. With the usual techniques (fixation in osmic acid and embedding in methacrylate) the number and aspect of these granules is variable from one preparation to another.

They are abundant in some preparations but scarce in others. Their density is low and frequently they become concentrated in areas where the hyalomere has a lower density. Sometimes they form branching aggregates. In preparations fixed and embedded in the same way, but where the sections have been previously stained with uranyl acetate or potassium permanganate, these granules have the same aspect and distribution but with higher density (Fig. 11). At higher magnifications it is possible to recognize inside these granules some dense particles of 30 A. In some preparations almost all the platelets have large quantities of these granules but in others prepared in the same way they are scarce.

The presence of the 180–200 A. granules is constant in the sections of platelets fixed with potassium permanganate, embedded in Araldite, and stained with uranyl acetate (David-Ferreira and David-Ferreira, 1962). With this technique the aspect of these granules is always more regular (Fig. 12). They are spherical, about 180 to 200 A. in diameter, very dense, and clearly distinct from each other. They have two types of distribution in the hyalomere: (a) in small groups between the granulomere elements; (b) in groups of hundreds of granules in peripheral zones of the platelets (Figs. 8, 13).



FIG. 11. Normal human platelet. Same technique as in Fig. 5. Between the platelet components are seen two drum-stick granules and 190-A. granules (glycogen). $\times 76,000$.

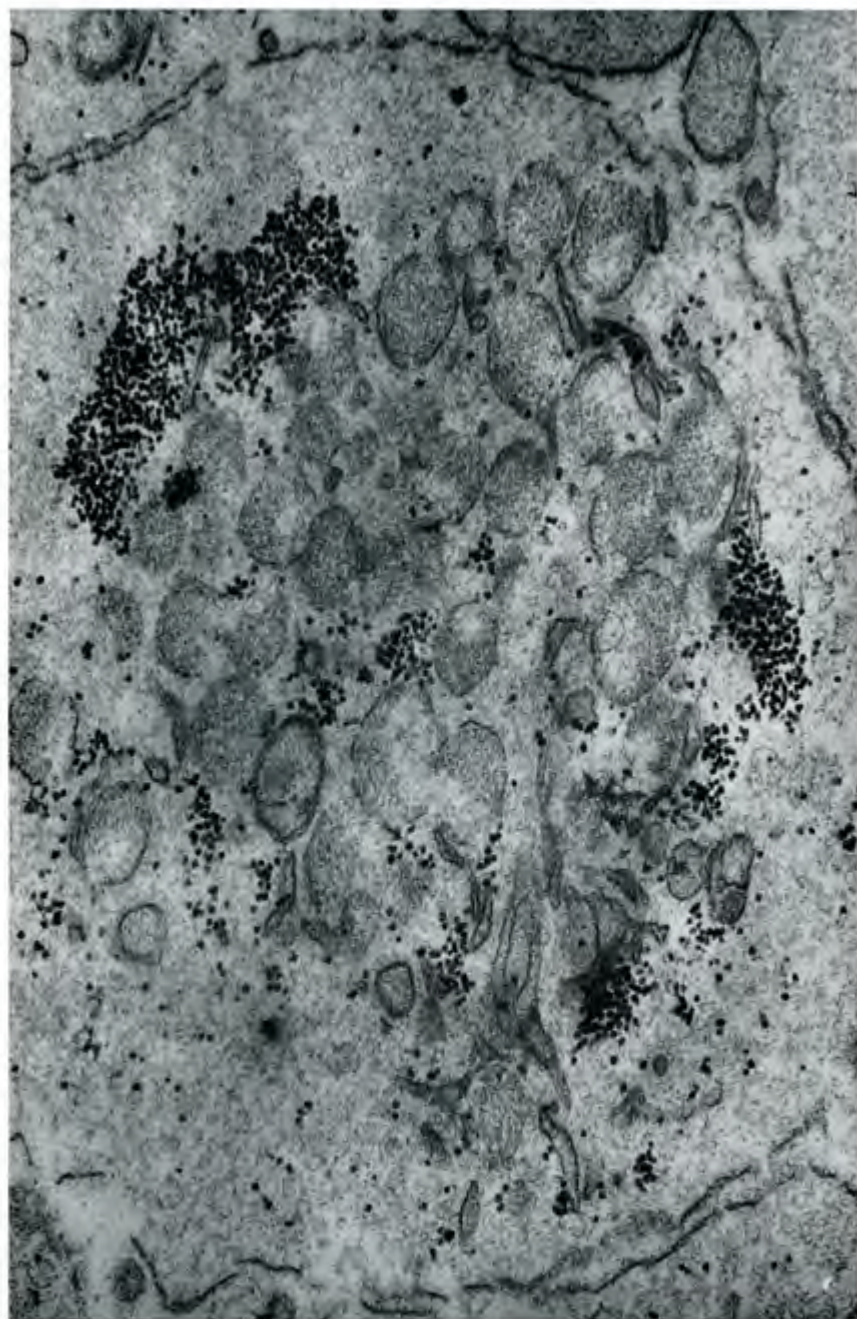


FIG. 12. Normal human platelet. Fixation in potassium permanganate; embedded in Araldite, stained with uranyl acetate. 180–200 Å. granules (glycogen) observed isolated or in groups are dispersed between the dense granules. $\times 45,000$.



FIG. 13. Normal human platelet. Same technique as in Fig. 12. The different components of the platelet granulomere are seen: the dense granules, mitochondria, vesicles, tubules, and groups of glycogen granules. $\times 45,000$.

In a previous study (David-Ferreira and David-Ferreira, 1962) it was concluded that they have the same characteristics as the glycogen granules described in the liver and other organs by several authors (Drochmans, 1960, 1961; Milonig and Porter, 1961; Revel *et al.*, 1960). Their density is lower in sections



FIG. 14. Diagrammatic representation of a normal human platelet: α —dense granules; β —mitochondria; γ —clear elements (vacuoles, tubules, microvesicles); δ —cytosome; ϵ —glycogen granules.

of material fixed with methacrylate, and they tend to adhere and form branching aggregates. In sections of material fixed with potassium permanganate and embedded with Araldite they retain their individuality and become denser after staining with potassium permanganate or uranyl acetate.

With the optical microscope we have observed in films of human blood, after the McManus technique, two types of staining: platelets with a hyalomere diffusely stained, and others with some granules intensely colored. After salivary

digestion the staining reaction is not observed. Probably after the McManus technique the platelets presenting a diffuse staining correspond to those that under the electron microscope have dispersed granules and those with stained corpuscles correspond to the others with aggregates of granules.

The conclusion that the 180–200 Å granules represent the platelet glycogen agrees with the work of Jean and Gautier (1961). These authors have demonstrated under the electron microscope with several "staining" techniques the presence of glycogen in these elements. More recently Jean and Racine (1962) found that after incubation in diastase the 180–200 Å granules lost their "staining" properties. According to these authors the platelet glycogen is formed in the megakaryocytes.

The existence of glycogen in the platelets has been previously demonstrated by several authors with histochemical or biochemical techniques, but the results are contradictory (see Maupin, 1961). The data recently obtained with electron microscopic studies support those who have described glycogen in the platelets and give precise information about its localization and distribution.

Concerning the observation of ribosomes in the hyalomere of normal platelets the results are contradictory. After fixation in osmic acid and inclusion in methacrylate some authors described the 150 Å granules but others have not observed them. It is possible to explain the different opinions if we admit that some of the granules described as ribosomes are glycogen granules.

iii. Dense Granules. Another type of small particle observed in the platelet hyalomere has been described by Schulz and Hiepler (1959). They are small dense granules with a diameter of approximately 120 Å. In their opinion these granules represent the dispersion of the matrix of dense granules (α -granules) in the hyalomere. For that reason they called them "liberalisierte α -substanz." The observations of these authors have not yet been confirmed and some of these granules are similar to the glycogen.

The review of the studies with the electron microscope on platelet morphology permits the conclusion that in spite of the fact that some points need to be clarified, the use of the electron microscope has already brought us a detailed image of platelet ultrastructure (Fig. 14).

III. Submicroscopic Morphology of Pathological Blood Platelets

A. BEFORE THE INTRODUCTION OF ULTRATHIN SECTIONS

Before the introduction of ultrathin sections the studies on the morphology of pathological platelets were numerous, not only in blood disorders but also in other pathological conditions. The review of the literature in this field is particularly difficult because of the nomenclature problem. As Braunsteiner and

Pakesch (1956) said, "the terminology of qualitative platelet disorders is controversial and confusing. Almost all possible terms have been interchanged for all possible diseases which were often vaguely determined." In our references we have utilized the designations employed by the authors, in order to avoid a supplementary confusion.

With the different techniques employed in this first period, the platelets were observed as a whole, and the orientation of the studies was based on the modifications of two of the platelet properties: pseudopod formation and their spreading ability.

Based on the alterations of these properties Braunsteiner (1955) and Braunsteiner *et al.* (1953, 1954a, c) defined a primary platelet disorder, thrombocytoasthenia, characterized by a defective pseudopod formation and lack of spreading. It is a congenital hemorrhagic disease; the patients have normal platelet numbers and normal coagulation factors. Identical observations have been reported by Alatas and Ulutin (1954).

According to Braunsteiner (1961) "the disease must be inherent to the platelets, since normal platelets in plasma or serum of the diseased person behave normally, whereas pathological platelets in normal plasma or serum remain pathological."

Alteration in pseudopod formation, defects in aggregation, and diminution in the number of granules have been described by Brüster and Sachsse-Klinke (1959) in a case of Glanzmann-Naegeli, a disease also studied under the electron microscope by Marx and Köppel (1957).

Another hemorrhagic disease studied by Braunsteiner and associates in 1955 and 1956 (see Braunsteiner, 1961) is a thrombocytopathia characterized by diminution or diminished release of factor 3. Under the electron microscope they observed platelets with fewer granules, but the pseudopod formation is normal and the spreading is normal or excessive. Similar observations are presented by Riddle and Rebeck (1958) and by Rebeck and associates (1959, 1961).

Defects in pseudopodial formation and in platelets spreading have been described also in the thrombocytopathia of Willebrandt-Jürgens, one of the diseases in which the platelets have been more frequently examined with the electron microscope (Sauthoff, 1951, 1952; Alatas and Ulutin, 1954; Igarashi, 1957; Brüster, 1959; Raccuglia and Neel, 1960). Other disorders with alterations in pseudopodial formation or in spreading are: the essential thrombopenia (Braunsteiner and Febvre, 1950a; Braunsteiner *et al.*, 1954c), leukemia (Braunsteiner and Febvre, 1950a; Gürtürk and Alatas, 1955; Igarashi, 1957), idiopathic thrombocytopenic purpura (Braunsteiner *et al.*, 1954c; Rebeck *et al.*, 1959), Waldenström's macroglobulinemia (Braunsteiner *et al.*, 1954b, c), lymphogranulomatosis (Sauthoff and Landschütz, 1951), anemias of hypochromic type,

nephritis, hepatitis (Igarashi, 1957), and different types of anemias (Abdoullaev *et al.*, 1957; Rebuck *et al.*, 1959; Riddle *et al.*, 1960).

The results obtained with the spreading techniques have given some information about platelet pathology; however, as in almost all the disorders studied, the alterations described are not specific and the data obtained adds little to our knowledge of their characteristics.

B. AFTER THE INTRODUCTION OF ULTRATHIN SECTIONS

In studies reported after the introduction of ultrathin sections attention has been focused on the ultrastructural alterations of the platelets. The first work in that direction was reported by Hemmeler (1958) who observed a remarkable decrease of the dense granules in a case of thrombocytopathia. He also noticed a decrease of the azurophilic granules with the optical microscope and a diminution of factor 3. He suggested the probable localization of the factor 3 in the dense granules.

The same year Schulz and associates (1958) described in several cases of Willebrand-Jürgens from the Aaland Islands a special type of granule, the drum-stick granules ("trommelschlegelgranula"). These authors believe that drum-stick granules are characteristic of that disease. They also find that the dense granules are smaller and less dense, the vacuoles are bigger, and mitochondria and cytosomes are rare. Some years later the same authors in collaboration with Eriksson (Eriksson *et al.*, 1961) described in the same disease drum-stick granules and dense granules bigger than in normal platelets. The other granulomere components are normal.

As we have mentioned in the second section of this article, drum-stick granules are observed in several different pathological conditions (Jean, 1961; Jean *et al.*, 1963a) and also in normal human platelets (David-Ferreira, 1961b). For these reasons they cannot be considered as characteristic of the Willebrand-Jürgens thrombopathia.

In the Glanzmann-Naegeli thromboasthenia, Gross and associates (1960) found alterations in the mitochondria and an increase in number and dimensions of the vacuoles. Loehr (1960), in the same disease, described mitochondrial swelling and a decrease in the dense granules. More recently Jean (1961) and Marx and Jean (1962) in several cases of Glanzmann-Naegeli reported: platelet anisocytosis, hypervacuolization, reduction in the mitochondria number or a mitochondrial swelling, and platelet steatosis. According to Marx and Jean (1962) none of these individual alterations can be considered characteristic of the disease but they represent an alteration of the platelet population.

In a study on the distribution of lipid droplets in normal and pathological platelets Jean and associates (1963b) also concluded that in platelet disorders

the proportion of lipid droplets is significantly higher, especially in congenital platelet diseases.

Other disorders where ultrastructural alterations of the platelets have been reported are the Wiskott-Aldrich syndrome (Gelzer and Gasser, 1961) and leukemia (Haguenau *et al.*, 1963). Gelzer and Gasser (1961) in the Wiskott-Aldrich syndrome described a decrease in the clear elements. In different types

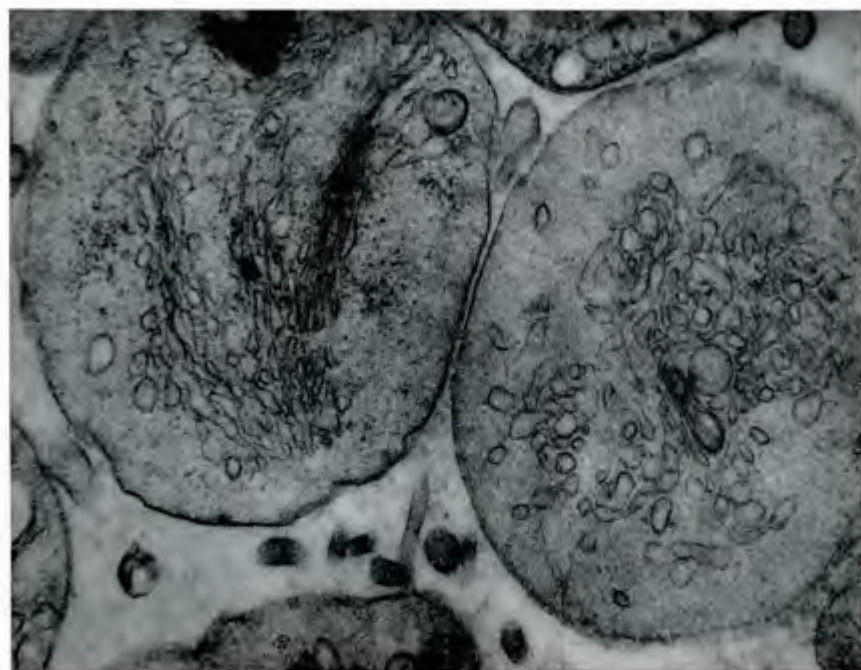


FIG. 15. Platelets: human leukemia, showing hypertrophied endoplasmic reticulum. $\times 39,000$. (Courtesy of Dr. F. Haguenau.)

of leukemia Haguenau and associates (1963) described a remarkable development of the vesiculotubular system (Fig. 15), an increase in the number of ribosomes, and sometimes the presence of organized ergastoplasm.

The review of the literature on the pathology of the platelets shows that up to now none of the ultrastructural alterations described can be specifically considered as characteristic of any disease. Also, the data obtained by quantitative analysis of the alterations found in ultrathin sections cannot be accepted without reservation. However, the combination of the ultrastructure techniques with fractionation and cytochemistry is one of the promising fields to be explored in the future.

IV. Submicroscopic Aspects of Platelet Physiology

A. IN COAGULATION AND HEMOSTASIS

The most important physiological functions of the blood platelets are related to their role in coagulation and hemostasis. The evidence of their intervention in these processes is partially based on data obtained by morphological methods. In agreement with these studies the coagulation of the blood is preceded by the aggregation and disintegration of the platelets. This process of clumping and fusing of the platelets into amorphous masses is named *viscous metamorphosis* and was described for the first time by Eberth and Schimmelbusch in 1886. More recently the term *viscous metamorphosis* or *platelet metamorphosis* has

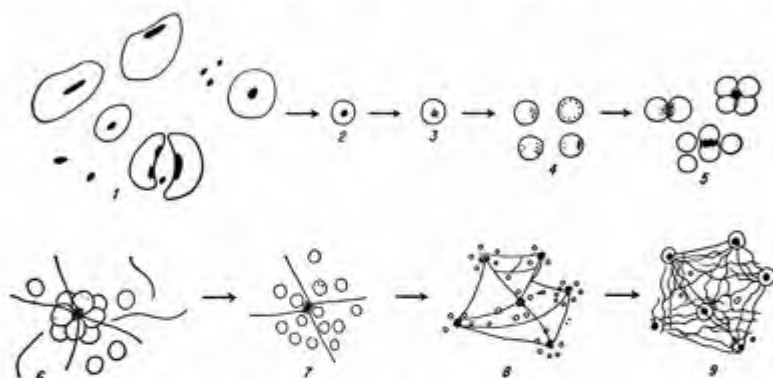


FIG. 16. Diagrammatic representation of the intermediate stages of viscous metamorphosis. (Courtesy of Dr. Rosenthal.)

been used to describe all platelet changes during clotting of the blood (Sharp, 1961).

Under the optical microscope these modifications have been observed in platelet-rich native plasma or in citrate plasma after the addition of calcium chloride. According to Setna and Rosenthal (1958) the following stages are observed (Fig. 16): The platelets become spherical and their granulomere material moves eccentrically to the platelet margin. This is the *signet ring form*. Then groups of platelets clump together by their granulomeres and form the *rosette* pattern, the center of which is formed by the granulomere material of several platelets. The *rosette petals* are represented by the hyalomeres. The fibrin strands appear between the platelet aggregates and some are adherent to the granulomere material.

Alterations of blood platelets during clotting have been studied since 1939 under the electron microscope. In these studies attention has been focused on platelet aggregation, hyalomere and granulomere modifications and their evolution, formation of the fibrin strands and their relation to the platelets. In the

beginning these transformations were observed in preparations of isolated platelets directly or after shadowing.

The earliest work on this subject, published by Wolpers and Ruska (1939b) in 1939, described, during clotting (*in vitro*), the formation of vacuoles and pseudopods, progressive hyalomere destruction, and the aggregation of fibrin strands to the rest of the granulomere. Ten years later Braunsteiner and Febvre (1949), also in preparations of human blood, observed after platelet aggregation the disintegration of the hyalomere into small granules. By ultracentrifugation they prepared a platelet microsome fraction which possessed thrombokinase activity. According to Braunsteiner (1951) thrombokinase activity is associated with the small granules released during the hyalomere disintegration. The same authors (Braunsteiner and Febvre, 1950b) interpret the platelets as centers of the clotting process.

Bloom (1955) also observed during coagulation, after the hyalomere disintegration, a large number of granules, 100–250 m μ in diameter, between the radiating strands. This author also considers the blood platelets as supporting centers for the fibrin network (Fig 17). In the vicinity of disintegrated platelets he observed short chains of spherical structures 150 to 300 A. in diameter, which he interpreted as molecules of fibrinogen in the process of aggregation to form fibrils of fibrin. These fibrils aggregate and form the fibrin fibers.

The action of anticoagulants in platelet aggregation has been studied by Jürgens and Braunsteiner (1950). They concluded that in the presence of anticoagulants fibrin on the surface or in the vicinity of the aggregates is not observed.

DeRobertis *et al.* (1953) described, during clotting, that the platelets closely bound to the fibrin are disintegrated and those not related with the fibrin are intact. These authors reproduced platelet disintegration by the addition of small amounts of thrombin. According to DeRobertis (1955), in the early stages of coagulation "the morphologically intact platelets act as centers which, by an unknown mechanism, orient the polymerization of fibrinogen taking place in the surrounding medium. This mechanism apparently occurs at the surface of the platelet and does not take place in platelets undergoing disintegration."

According to Aleksandrowicz and associates (1954) in fibrin formation it is the granulomere and not the hyalomere that has the important function. The same authors (Aleksandrowicz *et al.*, 1957) also described the vacuolization of the platelets and the destruction of their plasma membrane during viscous metamorphosis.

Utilizing the earliest techniques several other authors (Haydon, 1957; Hutter, 1957; Morita and Asada, 1957; Igarashi, 1957) have given descriptions of the transformations suffered by the platelets during clotting. Also, Köppel in 1958

revised the literature and gave a detailed description of the transformations suffered by the platelets during viscous metamorphosis. He observed the formation of bridges (150–400 Å. in width) between the platelets and evidence of a protoplasmic fusion during the aggregation.



FIG. 17. Low magnification micrograph of the clotting process as seen before the introduction of ultrathin section techniques. Fixation in buffered osmic acid; shadowed with Pt-Pd. $\times 6450$. (Courtesy of Dr. G. Bloom.)

More recently the morphologic modifications of the platelets during clotting have been studied in ultrathin sections and some new data have been obtained.

One of the first transformations noticed under the optical microscope, after the addition of calcium chloride to a citrated platelet-rich plasma, is the appearance of platelets with the granulomere collected at one point of the plasma membrane (*signet ring form*). With the same experimental conditions, in the thin

sections, under the electron microscope, the signet ring forms may also be recognized (Fig. 18). However, in this condition their observation is less frequent because this aspect depends on the plane of section. Another early transformation noticed is the aggregation of the neighboring platelets.

According to Rodmann and associates (1961, 1962, 1963), the earliest changes observed in platelets from recalcified plasma (10 minutes after recalcification) were: alterations in the platelet shape and concentration of the granulomere in the center of the platelets. They also noticed the formation of small aggregates of platelets and between them some fibrin strands. The platelet aggregates become larger and fibrin appears in greater amounts. Thirteen minutes after recalcification two types of aggregates were observed. In one, the more frequent, the limiting membranes are easily identified but it is difficult to recognize the granular elements. In the other type two different zones are recognized: a central one, with poorly defined granular material and without limiting membranes, and a peripheral zone with saclike protrusions partially limited by membranes. Strands of fibrin are observed within or adjacent to these masses (Fig. 19). Twenty-six minutes after recalcification, in the partially retracted clot, Rodman *et al.* (1963) described masses of platelet aggregates with a central material of no identifiable structure and peripheral saclike protrusions with intact limiting membranes. Between the masses strands of fibrin were observed.

The structural changes of the platelets during viscous metamorphosis have also been studied with the phase and electron microscopes by Castaldi *et al.* (1962). These authors described the aggregation of platelets 15 seconds after recalcification. The platelet plasma membranes are intact but bridgelike processes are seen between the adjacent platelets. Forty-five to ninety seconds after the addition of calcium the plasma membranes of most of the platelets are intact but an intimate adherence is observed between the neighboring platelets. They are swollen and pale and the aspect of their granulomere is variable. In some the granules are not recognizable.

When the fibrin appears (160 seconds) in some intact platelets a crescentlike peripheral alignment of their granules is observed. Some gaps in the plasma membrane and a decrease in the dense granules is also noticeable. Castaldi *et al.* (1962) state that the dense granules are "rarely seen as viscous metamorphosis proceeds and may be extruded or consumed in this process." Fibrin appears in the medium "often quite unrelated to any platelet substance," but sometimes is "closely adherent to platelet membranes which appear thickened at the site of the attachment." In the same stage we also observed (David-Ferreira, 1963) the adherence of the fibrin strands to the platelet plasma membrane, but fibrin also adheres to the plasma membranes of erythrocytes or leucocytes which are occasionally present. The presence of fibrin within the platelets described by Kuhnke (1961) has not been confirmed.

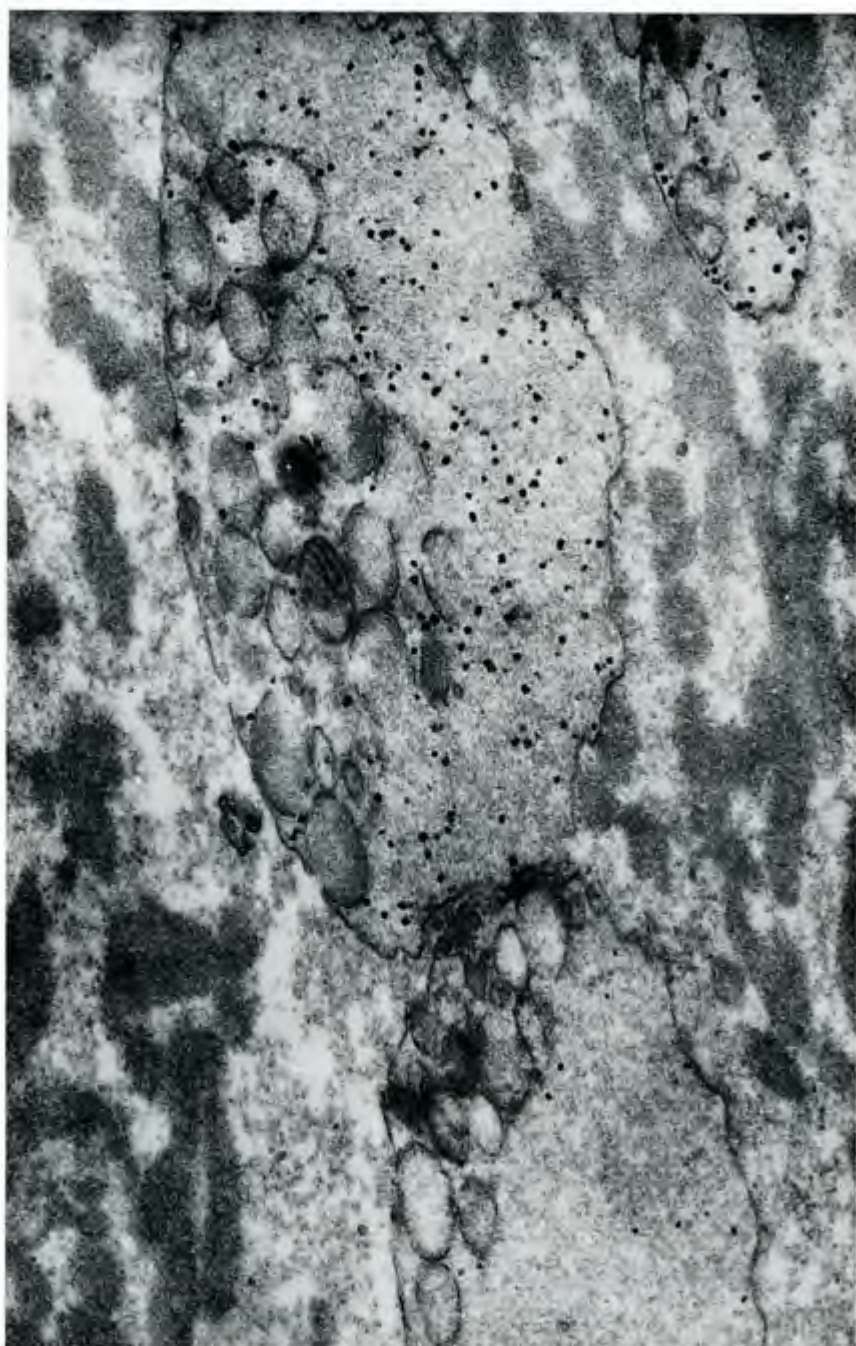


FIG. 18. Two platelets from a retracted clot in platelet-rich plasma. The dense granules and mitochondria are collected at one zone of the platelet margin ("signet ring" form). The small dense granules dispersed in the hyalomere are glycogen granules. $\times 38,500$.

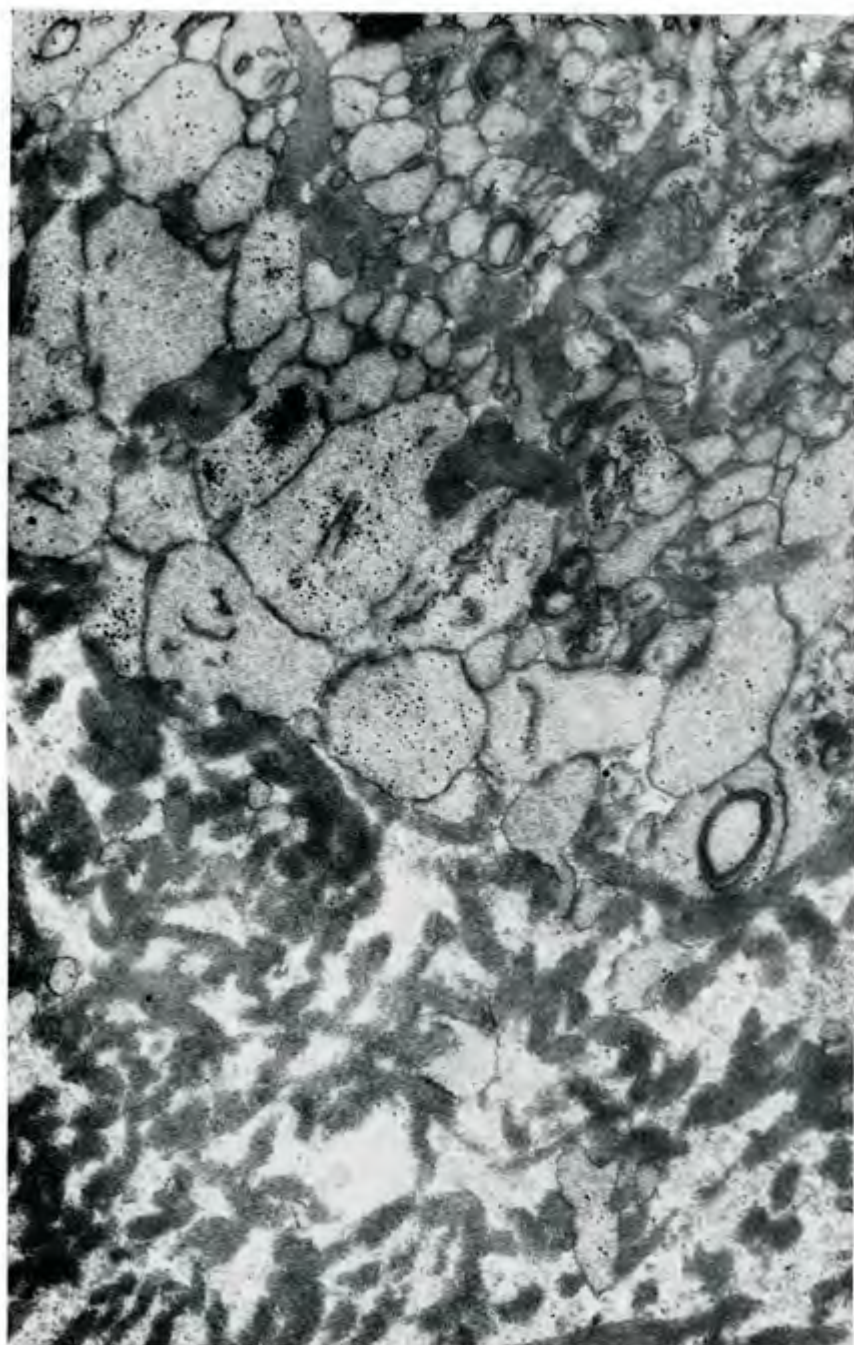


FIG. 19. Platelet aggregate 10 minutes after recalcification. Fibrin is observed between the platelets of the aggregate and adjacent to the aggregate. In some platelets groups of glycogen granules may be observed. $\times 19,750$.

The study of viscous metamorphosis induced by the addition of thrombin to a system containing no external fibrinogen has been made with the electron microscope by Parmeggiani (1961). Ten seconds after the addition of thrombin this author observed modifications in the densities of the platelets and the formation of small aggregates. After 60 seconds he noted the disintegration of granules inside the platelets. To Parmeggiani (1961) this "most likely explains the liberation of pharmacologically active substances, such as serotonin, from platelets during viscous metamorphosis." Some of the platelets do not participate in the process and thus conserve their individuality.

Recently the ultrastructure of viscous metamorphosis has been analyzed by Hovig (1962) after the addition of adenosine diphosphate or a saline extract of tendons of platelet-rich plasma to suspensions of washed platelets. In the aggregates induced by addition of small amounts of thrombin the fibrin appears only in the periphery of the aggregates and in the platelets a decrease of the dense granules was observed. The ultrastructure of these aggregates was similar to the hemostatic plug formed *in vivo* (Kjaerheim and Hovig, 1962). In the thrombin-produced aggregates of washed platelets, no visible fibrin was observed between the loosely packed platelets. This observation confirms the findings of Parmeggiani (1961). In the aggregates induced by extract of tendons, marked structural changes in the platelets were observed but the plasma membrane was preserved. No fibrin strands have been seen in this experiment.

In a platelet-enriched plasma recalcified and fixed at 15-second intervals Scott and associates (1962) found progressive degranulation, increasing vacuolization of the hyalomere, and disappearance of all platelet structures. The plasma membrane remained intact.

A comparative study of thrombi formed *in vitro* and *in vivo* and plasma clots has been made by Iseri and Benditt (1961). Their observations show that the thrombi are formed principally by aggregates of platelets with intact membranes but closely apposed. Their granulomere elements are not destroyed. Leucocytes are found closely adhered to the platelets and few fibrin strands are seen.

In the plasma clots Iseri and Benditt (1961) described a complete disruption of the platelets and abundant fibrin associated with the platelet masses.

Iseri and Benditt (1961) state that the important factors in the formation of the thrombus "are those which cause platelets to adhere to endothelium, to each other and to leukocytes. The role of fibrin appears less conspicuous."

The ultrastructure of hemostatic platelet plugs provoked by transection of arterioles and venules has been examined by Kjaerheim and Hovig (1962) in the rabbit mesentery. In agreement with these authors the platelets from the blood stream adhere to perivascular connective tissue. The plugs were formed almost entirely by platelets in close contact, which were more or less homo-

geneous. The fibrin was found at the surface of the plugs, sometimes close to the surface of the platelets but never in their interior.

The data obtained after the utilization of ultrathin sections has brought new understanding to the morphological transformations during clotting. However, new research is needed before we can develop a morphological picture that represents entirely the physiological and biochemical image of this complex phenomenon.

Another approach to the study of the role of the platelets in clotting has been made by the combination of fractionation and biochemical techniques with electron microscopy. In that direction Johnson *et al.* (1959) observed under the electron microscope a granule-rich sediment prepared from platelet extracts treated in a sonic oscillator and ultracentrifuged. This sediment, in which is localized platelet factor 3 activity, is formed by granules of about 350 m μ in diameter. Smears of these granules observed after staining under the optical microscope show they correspond to the azurophilic granules. Based on this evidence these authors conclude that the platelet factor 3 is associated with the azurophilic granules.

The same year Maupin (1959) reported that the physiologic activity of factor 3 is contained in a fraction which, observed under the electron microscope, is almost completely formed by the dense granules. This conclusion supports the views of Fonio (1951) who has reported the localization of factor 3 activity in the granulomere.

A study under the electron microscope of fractions isolated by differential centrifugation from normal platelets has been made by Schulz and Hiepler (1959). In agreement with these authors, factor 1 is localized in the different components of the granulomere (dense granules, mitochondria, vesicles). Factor 3 is in the dense granules and factors 2 and 4 in the fraction corresponding to the platelet hyalomere.

The results obtained in the fewer studies combining fractionation, biochemical and physiological analysis, and electron microscopy encourage new research in this direction.

B. OTHER PHYSIOLOGICAL ACTIVITIES (ADHESIVENESS, PINOCYTOSIS, AND PHAGOCYTOSIS)

When the platelets are put in contact with foreign particles they adsorb these particles on their surfaces. This phenomenon, which is an aspect of their adhesiveness, has a physiological significance for some authors. In fact, by their ability to adsorb particulate material, the platelets can clear from the blood foreign particles in circulation. In 1926 Tait and Elvidge observed that after injections in rabbits of quartz particles, barium sulfate, carmine, or India ink these sub-

stances are rapidly swept out of the blood stream and simultaneously a fall in the number of circulating platelets is observed. The same observation has been reported recently by Salvidio and Crosby (1960). These authors, utilizing isotope-labeled platelets studied their disappearance from the circulation after administration of India ink; they concluded that the thrombocytopenia observed was due to temporary sequestration of the platelets in the reticuloendothelial system. According to Salvidio and Crosby (1960) the "platelets seem to play an important role in the defense mechanism of the animal organism to the invasion of foreign particles."

According to several authors (see Tocantis, 1938) the property of platelets to adsorb foreign particles also suggests the possibility that the platelets can be involved in the reaction of the organism to bacterial invasion. Levaditi (1901) has observed that *Cholera vibrio* injected into rabbits adheres to the platelets. This adhesion of microorganisms to platelets, named *platelet loading* (Tocantis, 1938) has also been described by Delrez and Govaerts (1918), who include the participation of the blood platelets in the process of elimination of microorganisms from the circulation.

Taniguchi *et al.* (1930), after experimental work with several bacteria, concluded that "in contrast to the phagocytosis of leucocytes which consume living as well as dead bacteria and cellular debris by intracellular digestion after engulfing them, the blood platelets first stick to the foreign substance and digest it by secreting ferment extracellularly." These authors named this function *peptocytosis*; they thought that "the platelets serve for the first line of defense against the foreign substances and that the leucocytes form the second." More recently in an experimental study on tuberculosis Copley and associates (1955, 1959; Copley and Baléa, 1956, 1960) noted as the first reaction to the intravascular infection of dried BCG, platelet agglutination and their adhesion to the bacteria. In agreement with those authors (Copley and Baléa, 1960) "ce phénomène extraordinaire," the platelet reaction "n'est pas provoqué uniquement par les mycobactéries, mais fait partie à un ensemble de réactions propres à l'organisme pour se défendre contre n'importe quel autre germe y compris, peut-être les virus, et pour se débarrasser le plus vite possible de toute invasion microbienne."

With the electron microscope, using the spreading and shadowing techniques, Bloom and associates (1955) studied human platelets after putting them in contact with quartz particles. They observed that the quartz particles adhere to the platelet surface almost in the chromomere region. In other experiments with rabbits, a decrease of circulating platelets is observed after the intravascular injection of quartz particles. They do not give great significance to these experiments because the amount of the platelets in the blood of the rabbit is quite variable. However it is possible that after the injection of quartz the platelets which pick

up particles are destroyed. They conclude that the platelets "may have an important function in the elimination of solid particles from the blood."

With the optical microscope the ability of the platelets to incorporate material from the milieu has been explored by some authors. In 1896 Ramon y Cajal noted that the *fusiform* cells of the frog have phagocytic capacity, and some years later Tait and Gunn (1918) observed that the thigmocytes of crustacean blood (*Astacus fluviatilis*) are highly phagocytic. But the *fusiform cells* and the *thigmocytes*, physiologically equivalent to the platelets (see Jolly, 1923), are morphologically very different—they are bigger and nucleated.

With human platelets the ability to phagocytose bacteria has been investigated *in vitro* by Fiorito (1924); they concluded that platelets do not have this capacity.

An active incorporation *in vitro* by platelets was demonstrated for the first time by Bessis and Tabuis (1955) under the phase microscope (Fig. 20). These authors observed that the spreading platelets can adsorb liquids from the milieu (*pinocytosis*).

During a study with the electron microscope (in collaboration with J. Horta) on the distribution of colloidal thorium dioxide in rabbit organs we observed Thorotrast granules inside the platelets in the capillaries of the lung. In agreement with that observation we concluded that the platelets *in vivo* have the capacity to incorporate foreign particles (David-Ferreira, 1961a). In Figs. 21 and 22, which show a platelet inside a blood capillary of the rabbit lung, some Thorotrast particles are seen adhering to the platelet membrane and others are inside tubules or vacuoles. In other experiments with human platelets put into contact *in vitro* with colloidal thorium dioxide (David-Ferreira, 1961b), an intense phagocytic activity of the platelets has been observed. Platelets which remain for a longer time in contact with the colloidal thorium dioxide show numerous vacuoles filled with that substance.

In Fig. 23, which shows sections of human blood platelets put previously in contact *in vitro* with Thorotrast, the property of the human platelets to incorporate these particles and, also, the incorporation mechanism is revealed. These observations have been confirmed by Schulz (1961), who has observed *in vitro* that the rabbit platelets incorporate colloidal silica put in contact with them.

We can agree that in the experiments of Bloom *et al.* (1955) mentioned before, a phenomenon of incorporation of quartz particles by the platelets took place also, but with the methods utilized at this period it was impossible to observe whether the particles were inside or outside. In our experiments with Thorotrast, because we worked with ultrathin sections, we could recognize that the particles were not only on the platelet surface but also inside the platelet vacuoles.

One interesting observation in relation to this problem has been made by Danon *et al.* (1959). They described, in sections of platelets previously put in contact with influenza virus, vacuoles with virus particles. They concluded that "the mechanism of incorporation of the virus particles into the platelets has not been elucidated." Also, Dalton and Moloney (1962) described the presence of virus

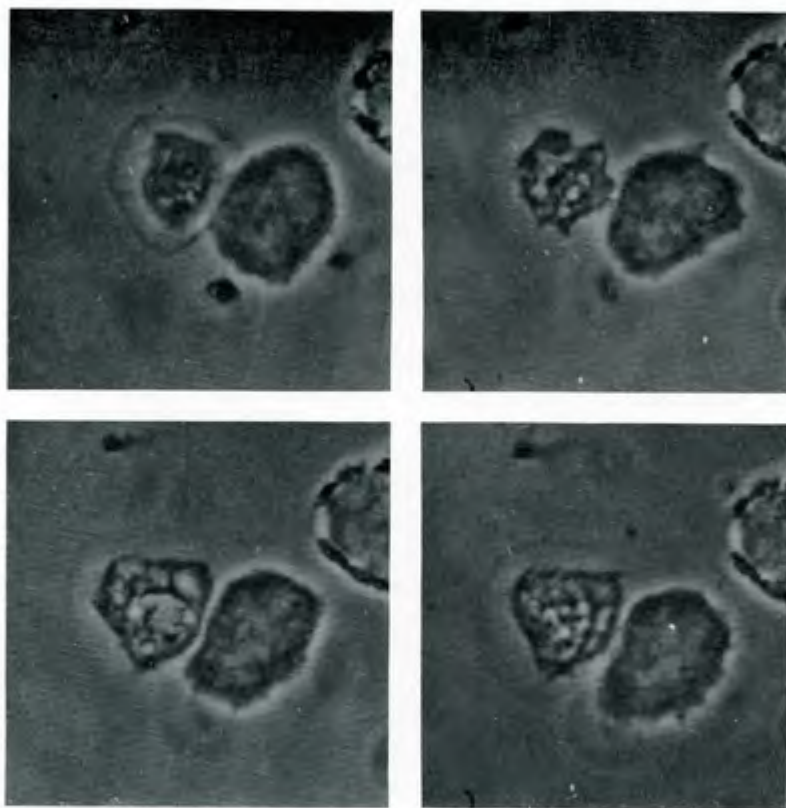


FIG. 20. Platelet pynocytosis seen under the phase contrast microscope. The formation of several vacuoles is visible in a platelet. (Courtesy of Dr. M. Bessis.)

on the surface of inside vacuoles in platelets of rats inoculated with a murine leukemia virus (Figs. 24). We can suggest that in these experiments, like those with the Thorotrast, *some* of the virus particles have been incorporated actively by the platelets.

We can also agree that the incorporation capacity of these elements can explain the "curious phenomenon of storage and release of pharmacologically active agents by platelets" referred to by Page (1958).

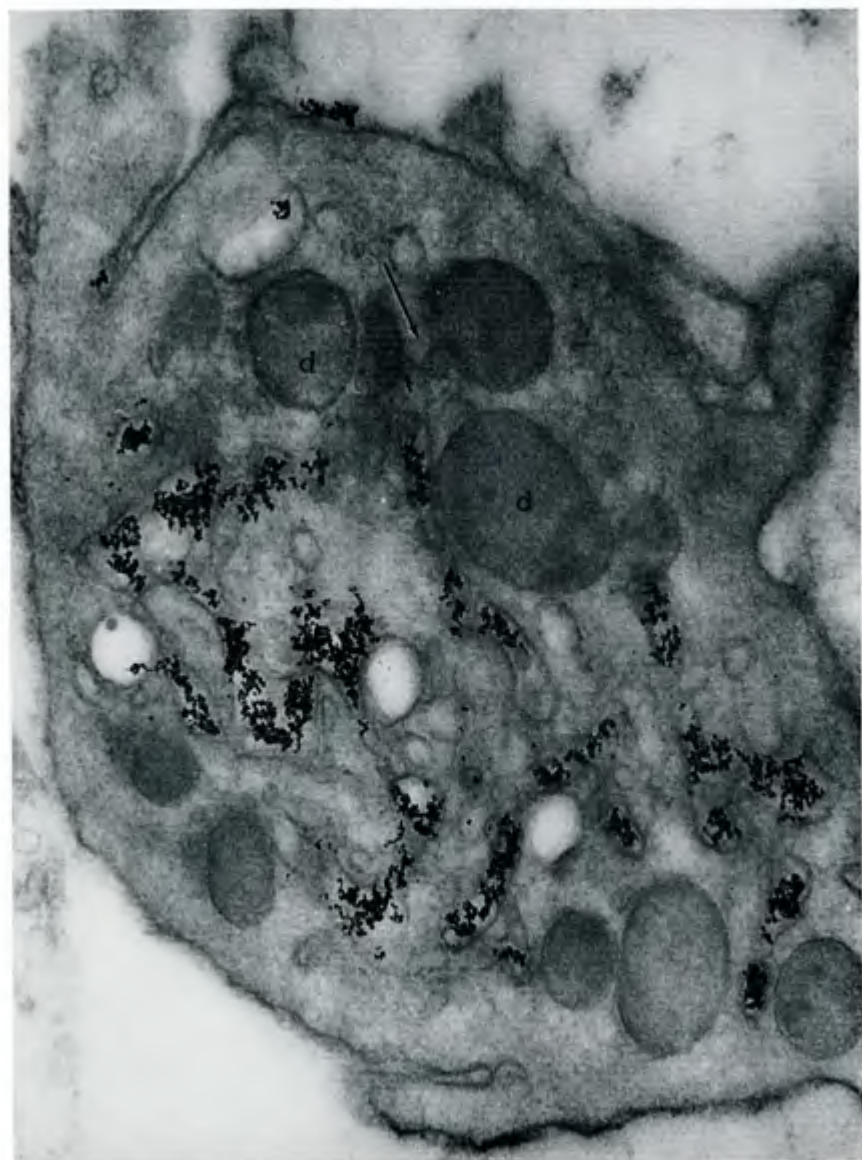


FIG. 21. Blood platelet in a lung capillary of a rabbit previously injected with colloidal thorium dioxide (Thorotrast). Fixation 24 hours after injection. Between the normal components of the platelets are observed some tubules and vacuoles with Thorotrast. d, Dense granules limited by a double membrane. Some have denser zones. At arrow small lobule in the surface of a dense granule. $\times 75,000$.

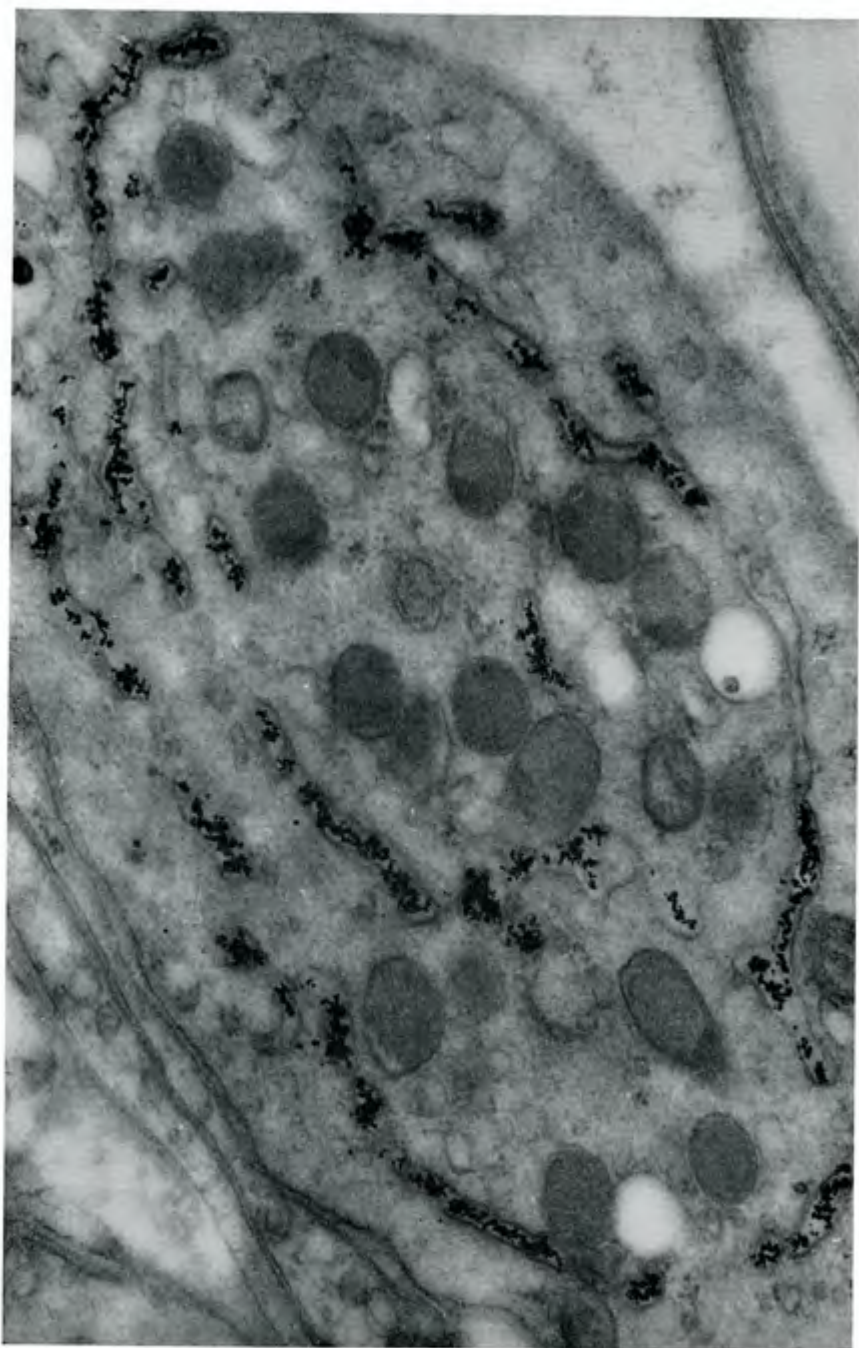


FIG. 22. Blood platelet in a lung capillary of a rabbit previously injected with Thorotrast. Fixation 24 hours after injection. Numerous tubules filled with Thorotrast whose disposition suggests a circulatory movement. $\times 75,000$.

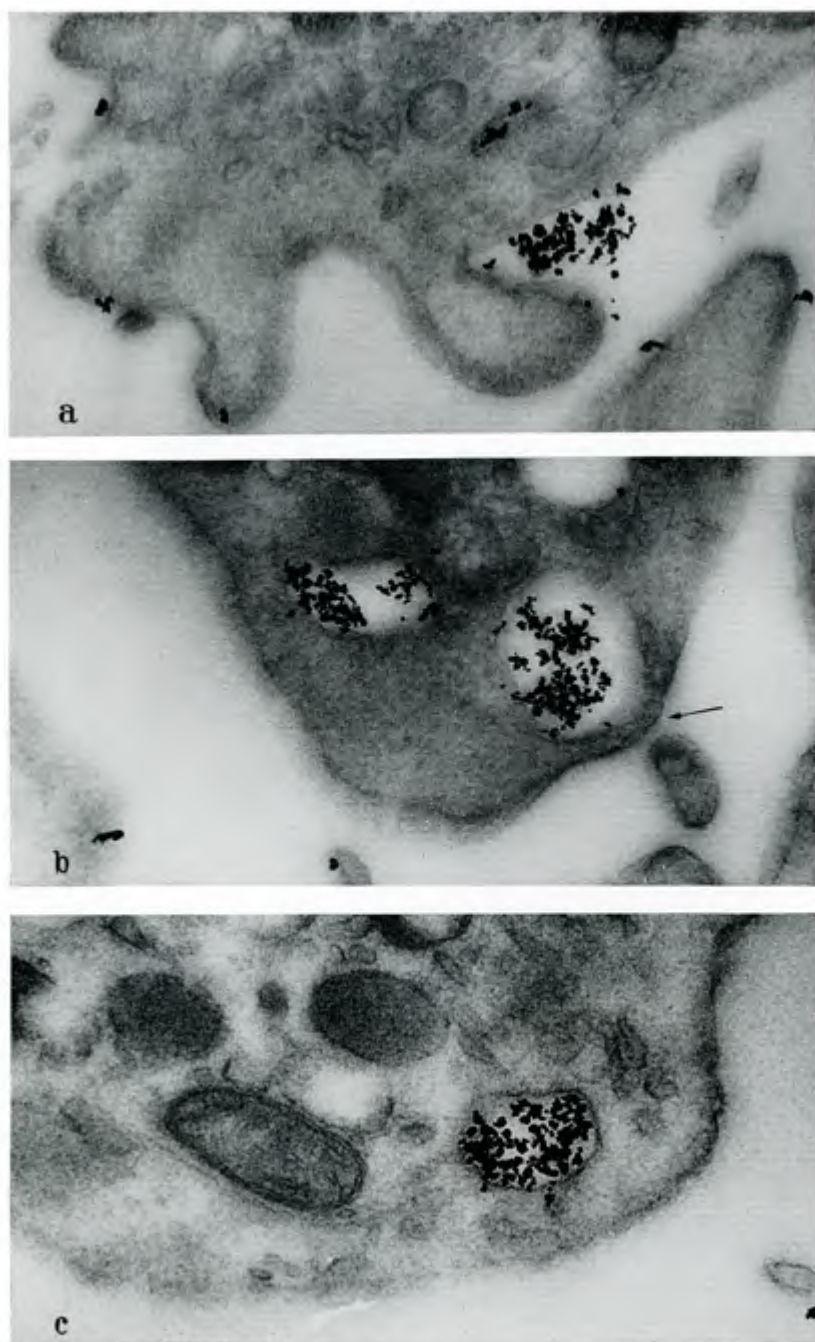
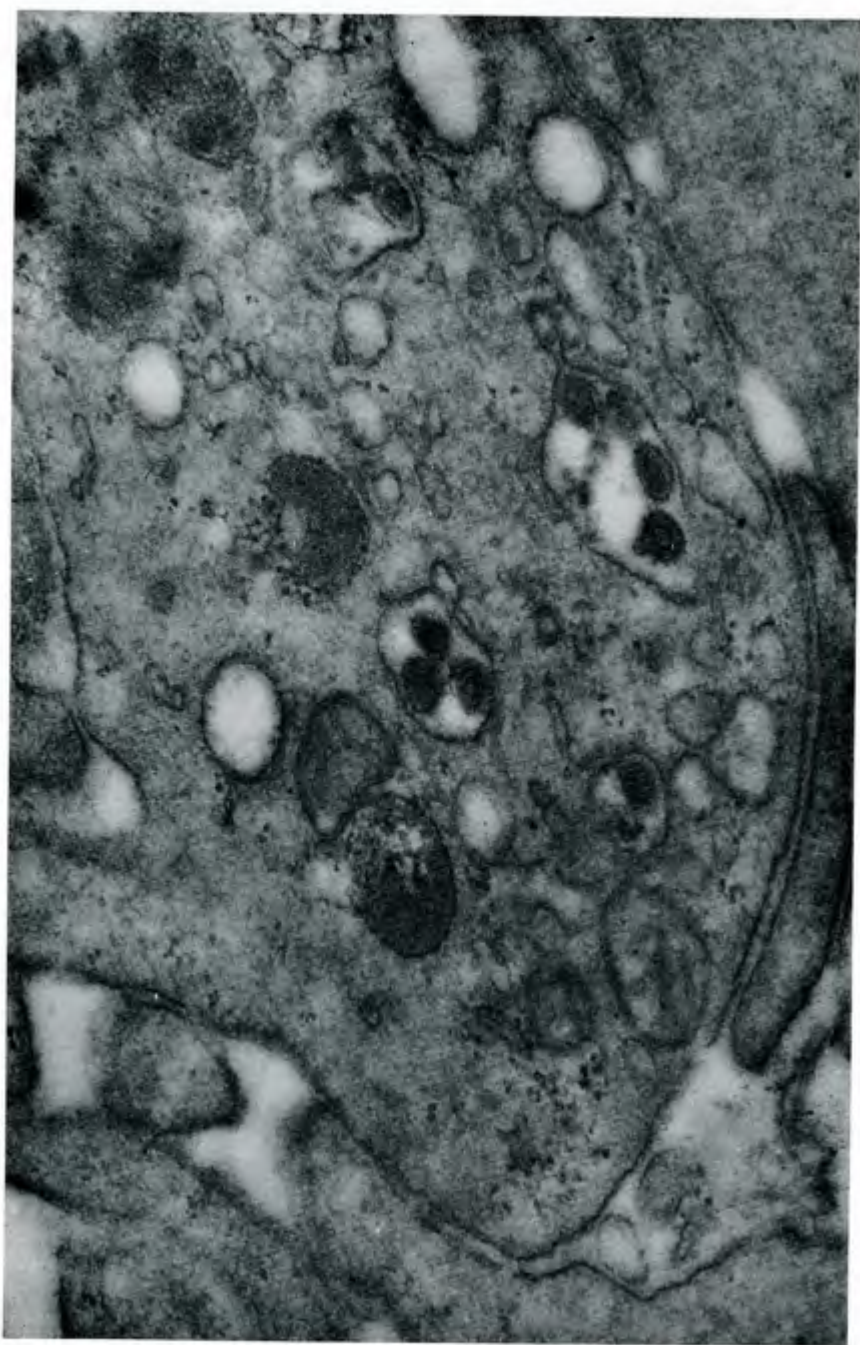


FIG. 23. Human blood platelets previously put in contact with Thorotrast. Aspects of the Thorotrast incorporation: a, pseudopod enveloping a group of particles; b, superficial vacuole with Thorotrast; the junction point is indicated by the arrow; c, vacuole already incorporated inside the platelet. $\times 75,000$.



V. Formation and Destruction of the Blood Platelets

For several years the origin of the blood platelets has remained an insoluble problem. In spite of the early work of Bizzozero and others, for whom the platelets are independent elements of the blood, some authors argued that they are destruction products of leucocytes or erythrocytes. To others they represent precipitates of disintegrated blood plasma or the fragmentation products of endothelial cells.

In 1906, for the first time, Wright described the formation of the blood platelets from the bone marrow megakaryocytes. This hypothesis is classified by Ramon y Cajal as audacious. Indeed, as Perroncilo (see Ramon y Cajal and Tello y Muñoz, 1956) said: "cuanto sabemos de la ontogenia de los tejidos, no muestra un solo hecho bien establecido de celula, en cuyo protoplasma se enjiendram elementos anucleados, absolutamente diferentes en morfologia estructura y actividade fisiologica del elemento anatomico progenitor."

As with all audacious hypotheses the Wright hypothesis has ardent opponents and defenders.

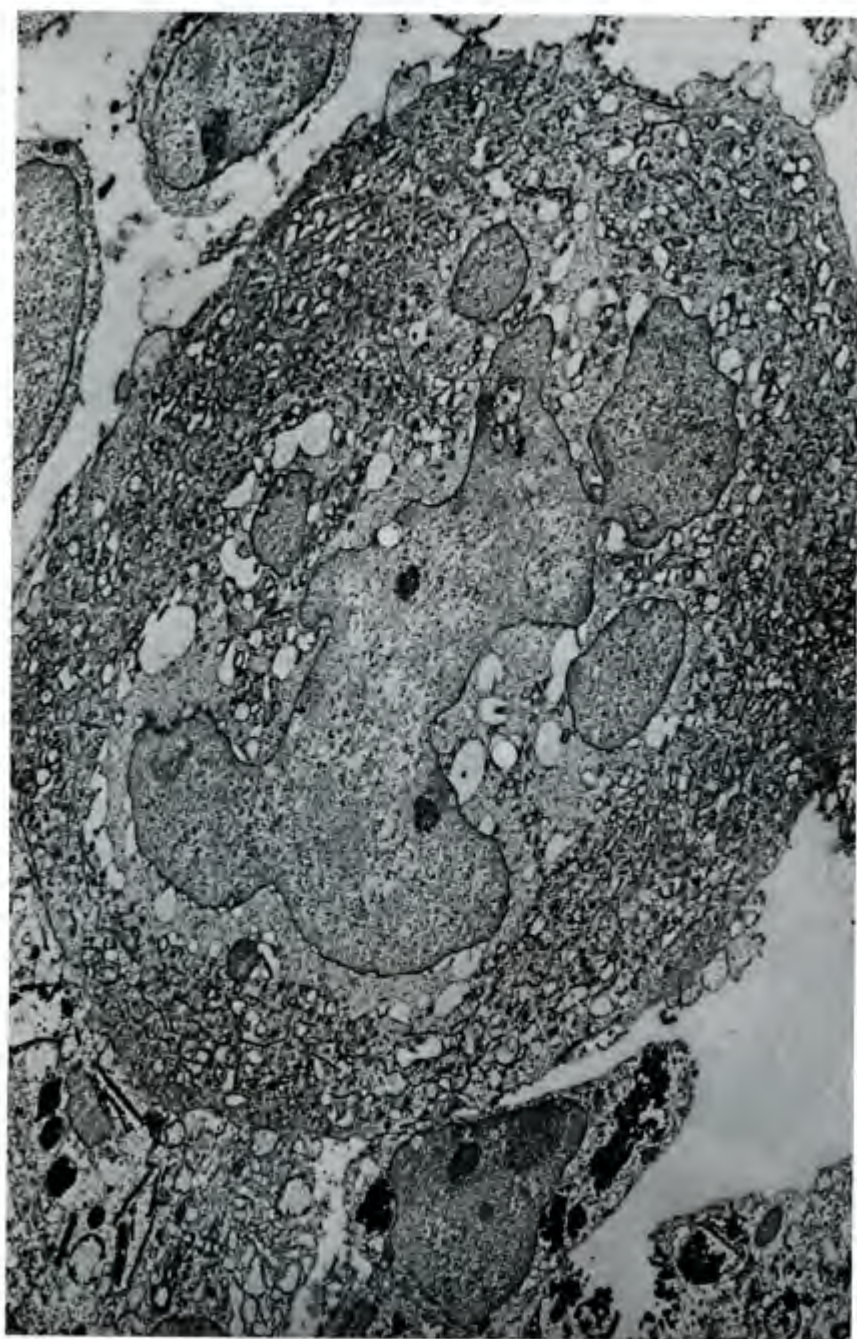
Based initially on the similarity between the platelets and the megakaryocyte cytoplasm and on pictures of the pinching off of portions of megakaryocyte cytoplasm into the vessels (*Wright pictures*), the Wright theory gained progressively new arguments and more defenders. Finally the formula: "the giant of bone marrow forms the dwarf of the blood" expresses the general opinion. In spite of this, however, in 1954 Bessis wrote: "Il faut cependant ne pas oublier que, par le moment personne n'a encore vu d'une manière indiscutable des thrombocytes se détachant 'in vivo' du cytoplasme des mégakaryocytes."

In 1956 in a moving picture film with the phase contrast microscope, Thiery gave a demonstration *in vitro* on the formation of the platelets from the megakaryocytes (Thiery and Bessis, 1956). In the same period the works of Humphrey (1955), Kinoshita and associates (1956), and Zajicek (1957) gave new arguments for the Wright theory.

Humphrey (1955) demonstrated with fluorescent antibodies that platelets and megakaryocytes have similar antigenic properties. In a moving picture film of rabbit bone marrow Kinoshita and collaborators (1956) demonstrated *in vivo* the formation of platelets from the megakaryocytes. On the basis of a histochemical study Zajicek (1957) concluded that the only possible origin of the platelets was the megakaryocyte.

Another important contribution to the study of this problem has been given

FIG. 24. Platelet from peripheral blood of Osborne-Mendel rat infected with the Moloney agent. Characteristic particles of the Moloney leukemia are present in vacuoles. $\times 70,000$. (Courtesy of Dr. A. J. Dalton.)



by electron microscopy. In this chapter the fundamental data obtained with this technique is summarized.

A. THE ULTRASTRUCTURE OF THE MEGAKARYOCYTE

The megakaryocytes are cells about 40 to 60 μ in diameter, but sometimes they can reach 160 μ . The designation of *giant cells*, also employed to name these cells, is well justified.

In sections of bone marrow of young animals the observation of the megakaryocytes with the optical microscope is very easy. Generally located near the blood vessels, they have a large multilobed nucleus with an irregular outline. In their cytoplasm the azurophilic granules are numerous and in the periphery of the cell they form small aggregates.

The first description of these cells with the electron microscope, still with the shadowing technique, was made by Bessis in 1954. He observed that the entire cell is covered by fine granules of uniform size and recognized on their surface small areas centered by aggregates of these granules.

With the ultrathin section technique the megakaryocyte ultrastructure has been studied by: Yamada (1955, 1957), Pease (1955, 1956), DeMarsh and associates (1955), Bessis (1956), Bessis and Thiery (1956), and more recently by Matter *et al.* (1960), Jones (1960), Schulz (1960), and David-Ferreira (1962).

The identification of megakaryocytes in thin sections of bone marrow is not difficult. Their shape and dimensions and the ultrastructure of their nucleus and cytoplasm are very characteristic (Fig. 25).

The nucleus, generally situated in the middle of the cell, is large and multilobed. In thin sections sometimes their lobes seem independent.

After the usual techniques (fixation with osmic acid and embedding in methacrylate) their nucleoplasm is homogeneous with darker masses correspondent to the nucleolus.

In the cytoplasm numerous oval granules are dispersed about 0.30 μ in diameter. They are limited by a distinct membrane and their matrix is homogeneous. These granules, named *large platelet granules* by Yamada (1957), are equivalent to the azurophilic granules observed with the optical microscope. Their ultrastructure is identical to the dense granules of the platelets.

Another granular structure observed in the megakaryocyte cytoplasm is the mitochondria. They are small and their structure is identical to the mitochondria

FIG. 25. Megakaryocyte from mouse bone marrow. A lobulated nucleus with small nucleolus is seen. In the cytoplasm two zones are recognized: a perinuclear, inner one and an outer zone with a developed membranous system (platelet demarcation membranes) and numerous granules. $\times 5200$.

of other cell types. They have the same size as the dense granules and sometimes are difficult to distinguish from them (Pease, 1956).

The megakaryocyte Golgi apparatus, usually located in the perinuclear zone, is formed by the same elements that are in other cell types. In their vicinity mixed with their vesicles some dense granules are observed. In agreement with Jones (1960) the precursors of the dense granules are formed by the Golgi apparatus.

In the cytoplasm of a mature megakaryocyte ribosomes and some ergastoplasm membranes are also described, but the prominent feature are the systems of paired membranes (Yamada's platelet demarcation membranes) that divide the megakaryocyte cytoplasm into small zones (1-2 μ) with an aspect identical to the platelets.

According to Yamada (1957), who confirmed the previous work of Heidenhain (1894), it is possible to divide the megakaryocyte cytoplasm into three zones: an inner zone surrounding the nucleus, a middle zone with almost all the cytoplasm and in which the platelet demarcation membrane system is located, and an outer zone formed by a narrow band of cytoplasm without cytoplasmic organelles. The outer zone, variable in size, is sometimes absent.

With the optical microscope several authors have reported (see Chaves, 1936) the observation of different types of blood cells (erythrocytes, leukocytes) inside the megakaryocytes. This is the basis for the hypothesis that the megakaryocytes have a phagocytic activity. To others these images are superposition images (Bessis, 1954).

In the thin sections studied with the electron microscope the observation of blood cells inside the megakaryocyte is frequent. This is the proof that the images observed with the optical microscope are not superposition images.

It is possible that the incorporation of the blood cells by the megakaryocyte is not a real phenomenon of phagocytosis but that the cells are involved by the megakaryocyte when this cell is in movement.

In a study on the distribution of colloidal thorium dioxide in organs of the mouse and rabbit (Horta and David-Ferreira, 1961), we examined under the electron microscope several sections of bone marrow. Some of the megakaryocytes observed in these sections always have small amounts of Thorotrast in the peripheral cytoplasm.

A remarkable phenomenon in the megakaryocyte cytoplasm has been described by DeHarven and Friend (1958, 1960) and Dalton *et al.* (1961; Dalton and Moloney, 1962). These authors have observed in megakaryocytes from the bone marrow and spleen of leukemic mice numerous virus particles within the channels formed by the platelet demarcating membranes (Fig. 26). Evidence of the viral multiplication in the megakaryocytes is also reported. From the biological point of view this fact is interesting, as Dalton said: "If these particles are

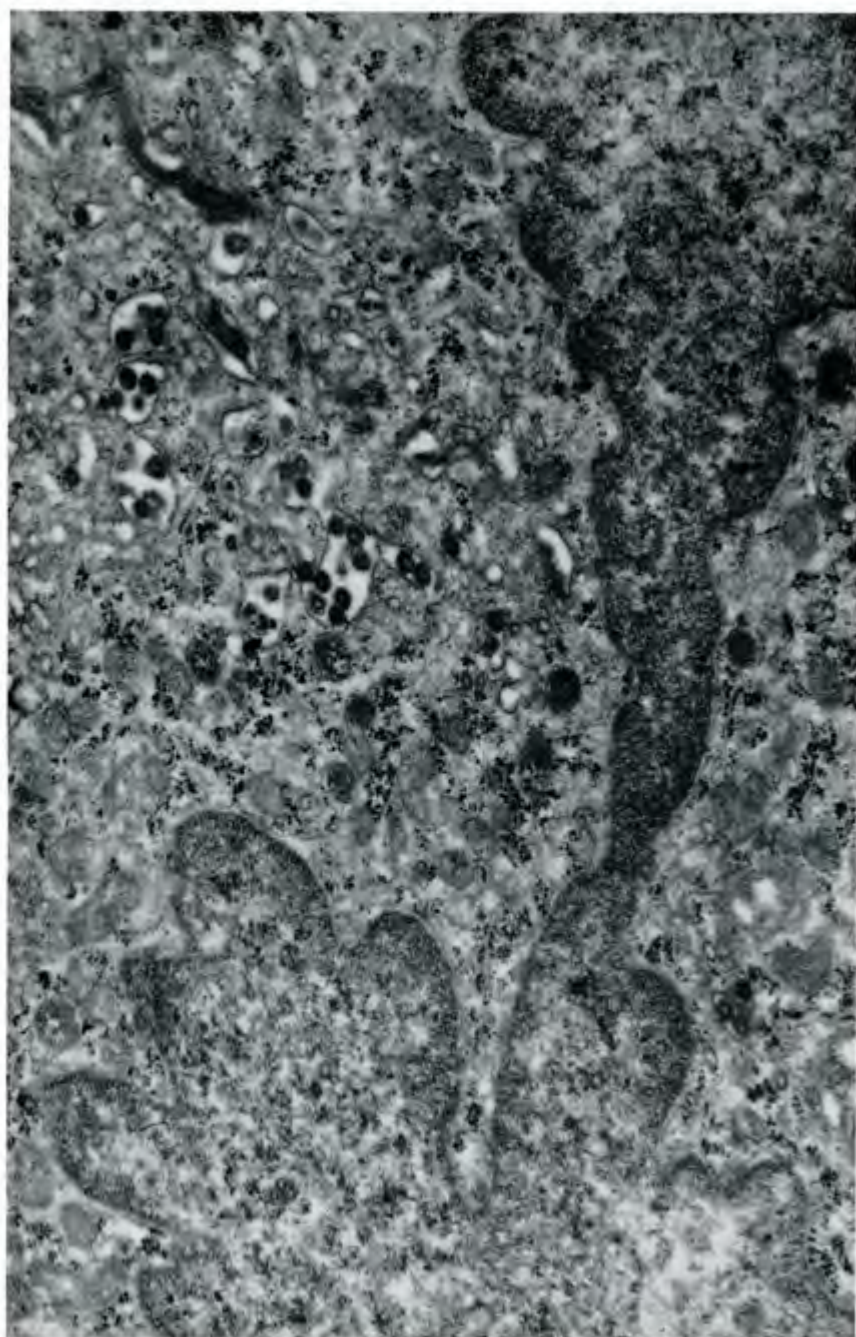


FIG. 26. Low-power view of part of a megakaryocyte from spleen of BALB/c mouse infected with the Moloney agent. Particles with electron-dense nucleoids are seen. $\times 35,000$. (Courtesy of Dr. A. J. Dalton.)

accepted as being the morphological representations of oncogenic agents, then these are examples of a non-malignant cell functioning as a site of replication of oncogenic virus."

B. THE FORMATION OF PLATELETS

The platelets are formed as a consequence of the development of the system of membranes in the megakaryocyte cytoplasm. To analyze this problem it is necessary to study the origin and evolution of these membranes during the megakaryocyte differentiation. With the electron microscope this problem has been studied by Yamada (1957), Bessis (1957), Jones (1960), and Schulz (1960). Yamada described four stages in the megakaryocyte evolution: pre-vesicular, vesicular, membranous, and residual.

In the prevesicular stage the cell has a relatively small cytoplasmic volume and a horseshoe-shaped nucleus. The Golgi complex and several centrioles are located in the "endoplasm" (portion of the cytoplasm limited by the nuclear lobes). In the "exoplasm" (portion of the cytoplasm between the nucleus and the cellular membrane) vesicles are dispersed. In these stages the cytoplasm does not yet contain specific granules. The prevesicular stage corresponds to the basophile megakaryocyte of Bessis (1957).

The vesicular stage, the granular megakaryocyte of Bessis (1957), is characterized by the appearance of numerous vesicular components in the cytoplasm. These vesicles arranged in rows of sheets delimit masses of cytoplasm.

Finally, in the membranous stage a greater number of vesicles and tubules are observed. This is the mature megakaryocyte.

According to Yamada's (1957) observations the vesicles coalesce and the double membrane system is formed. The platelet formation is intimately related to this system. Initially the double membranes only divide the megakaryocyte cytoplasm incompletely into small territories. With the membrane development the complete separation of the territories and the formation of the platelets results.

During the differentiation of the mature megakaryocyte another phenomenon observed is the formation of the dense granules. According to Jones (1960) they are related to the megakaryocyte Golgi apparatus and their formation "commences with the accumulation of a small electron dense core" in the center of a Golgi vesicle.

Electron microscopy has served as an excellent approach in an attempt to determine the mechanism of thrombopoiesis, but some aspects of the problem have not yet been explained.

What kind of mechanism determines the transformation of the megakaryocyte cytoplasm into the small territories of the platelets? What stimulus determines

the active approach of these cells to the blood capillaries where they release bits of their cytoplasm?

C. THE DESTRUCTION OF BLOOD PLATELETS

In agreement with the data presented by several authors (Maupin, 1954, 1957; Odell and Anderson, 1959) the life span of the blood platelets is estimated to be approximately 4 to 5 days. Where and why are the platelets destroyed?

It has been suggested, without complete proof, that the reticuloendothelial system has an important function in the removal of senescent platelets from the circulation and according to some authors the spleen has a primary function in their destruction. In some experiments carried out in the rabbit we have observed that the spleens of the animals injected with Thorotrast are completely filled with partially destroyed platelets filled with Thorotrast. Also, in other organs like the lung, liver, and kidney, platelets with Thorotrast are observed, but the great majority of these partially destroyed elements has been seen in the spleen. We have never found evidence of phagocytosis of platelets by macrophages.

At the moment we agree with Maupin (1954): "très probable l'intervention de la rate dans l'élimination des plaquettes vieillies mais elle n'est certainement pas exclusive."

VI. Summary and Conclusions

The use of the electron microscope in the study of blood platelets has provided new data concerning their morphology, physiology, and pathology.

With the new techniques the ultrastructure of the platelets has been analyzed and their components studied.

These small blood elements are limited by a membrane which ultrastructurally is identical to the cell membrane. In their hyalomere several granulomere elements are dispersed: the dense granules, sometimes with a drum-stick form; the mitochondria, small and with few cristae; the cytosomes; the clear elements (vacuoles, vesicles, and tubules); and small granules 150–200 Å. in diameter.

With the "staining" techniques, under the electron microscope, the presence of large amounts of glycogen granules dispersed or forming aggregates in the platelet hyalomere has been established.

Some studies have been made combining cytochemical methods with electron microscopy and further work in that direction is in progress.

The new techniques have also proved to be good methods for the study of physiology of the platelets. Platelet transformation during the viscous metamorphosis has been analyzed, and their relation to fibrin during clotting has been studied.

Combining fractionation, biochemical, and physiological techniques with

electron microscopy, new evidence has been obtained concerning the localization of factor 3 in the dense granules.

A clear demonstration of the phagocytic ability of the platelets (*in vitro* and *in vivo*) has been made. The implications of that property in physiology and pathology needs further exploration.

In the pathological field some new data for the characterization of platelet disease has been obtained with spreading and shadowing techniques and with ultrastructure methods.

Megakaryocyte morphology has also been analyzed under the electron microscope, and the mechanism of platelet formation has been established.

The comparison between the results obtained with the electron microscope and those obtained with the classic morphological techniques gives a clear picture of the progress achieved in the last few years. However, the possibilities of the new methods have yet to be completely explored, and "*We must be prepared, therefore, for many revelations regarding the intimate nature of the platelet*" (Tocantins, 1961).

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